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THE RATE OF METABOLISM OF CORTISOL IN A NORMAL HUMAN MALE*

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INTRODUCTION

A LIMITED amount of information has been published on the metabolism of cortisol in the normal human subject, using cortisol-4-C^{14} as tracer (1, 2). The present experiment was designed, not only to give insight into the over-all metabolism of the hormone, but to attempt to identify regions of metabolic activity through the study of turnover rates in the arterial, peripheral venous, and hepatic venous plasmas.

EXPERIMENTAL

As a means of tracing metabolic changes, a 420-microgram dose of cortisol-4-C^{14} which had been biologically synthesized from progesterone-4-C^{14} in our laboratory (3) was injected intravenously over a period of three minutes into a 24-year-old normal male subject weighing 71 Kg. The cortisol had a total radioactivity of 5 microcuries (μc.).

Indwelling catheters had been previously placed in the hepatic vein, the femoral artery, and the antecubital vein. From these catheters, 20-ml. samples of blood were taken simultaneously at fifteen minutes, thirty minutes, one hour, two hours and four hours after injection of the labeled steroid. Heparin was used as an anticoagulant in

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the sample tubes. Urine was collected at known intervals during the following three days; the feces were also saved during the first forty-eight hours.

The blood samples were immediately centrifuged and the plasmas extracted four times with equal volumes of a 4:1 ether-chloroform mixture. Two aliquots of the plasma extracts were plated and counted in a gas-flow counter. All counts were corrected for self-absorption of the sample.

In Figure 1 the radioactivities of the various blood samples are plotted as

$$\log \frac{\text{c/min./Kg. body wt. injected} \times 100}{\text{c/min./L. plasma found}} \text{ against time.}$$

The values in the arterial blood all fell very close to a straight line—the typical first-order decay curve. This was also true for the samples from the hepatic vein, except for the one-hour sample. In view of the consistency of the other points we have considered this one variation to be due to experimental error, although there is no direct proof of this assumption. The samples from the antecubital vein first tended to follow the slope of the arterial samples, but from one hour on, the points fell on a straight line with significantly less slope than either that of the arterial samples or that of the hepatic venous specimens. The slopes, zero intercepts, and calculated half-lives are listed in Table 1.

The packed cells from the one-hour samples and four-hour samples were hemolyzed and extracted with the ether-chloroform mixture. The

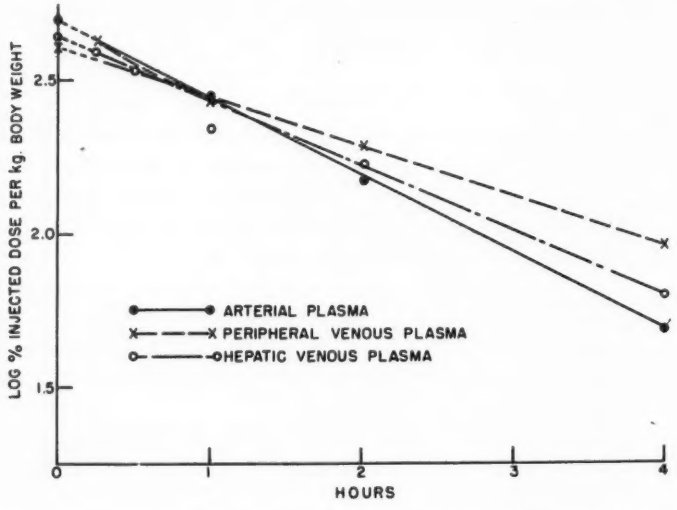

FIG. 1. Variation of chloroform-soluble radioactivity in plasma with time.

TABLE 1. DISTRIBUTION AND RATE OF TURNOVER OF CHLOROFORM-SOLUBLE ISOTOPE AFTER ADMINISTRATION OF CORTISOL-4-C^{14}

	Slope $\log \frac{\%\ \text{recov.}^*}{\text{hr.}}$	Zero time intercept		Half-life (min.)
		% recov.*	Apparent distrib. vol.† (%)	
Arterial plasma	−0.251	484	21	72
Hepatic venous plasma	−0.210	436	23	86
% Venous plasma 1st hour	−0.245	482	21	74
% Venous plasma, 1–4 hrs.	−0.158	398	25	114

* "% recov." $= \frac{\text{c/min./Kg. body wt. injected} \times 100}{\text{c/min./L. plasma found}}$

† Apparent distribution volume is the percentage of the body mass which would contain all the administered isotope at the level found in the plasma.

radioactivity in the extracts from the one-hour samples varied between 4 per cent and 8 per cent of the count in the plasma. Since this is approximately the proportion of plasma in such a cell layer, it was assumed that no significant penetration had occurred. There was no significant count in extracts from the four-hour samples.

The remainder of each of the ether-chloroform extracts from plasma was chromatographed on paper, using the benzene/55%-aqueous methanol system of Bush (4). Table 2 shows that although there was considerable radioactivity at the origin and in the region intervening between it and the cortisol area, the latter had considerably the

TABLE 2. DISTRIBUTION OF COUNTS AFTER CHROMATOGRAPHY OF PLASMA EXTRACTS

	Standard compounds*	Counts per min. for strips 1½ cm. wide × 3 cm. long														
		A† 15′	PV† 15′	HV† 15′	A 30′	PV 30′	HV 30′	A 60′	PV 60′	HV 60′	A 120′	PV 120′	HV 120′	A 120′	PV 240′	HV 240′
Origin		60	67	73	42	51	54	16	34	6	20	38	21	8	9	20
Strip 2		46	39	44	32	40	40	21	16	11	32	57	36	22	33	19
Strip 3		47	31	53	37	40	34	14	24	14	24	54	39	24	34	19
Strip 4	Cortisol spot	232	203	264	152	176	186	156	164	171	107	93	94	34	44	38
Strip 5		50	39	45	22	9	25	32	47	33	28	12	33	6	24	10
Strip 6	Cortisone spot	11	7	24	5	19	16	18	22	1	0	8	0	0	4	0
Strip 7		4	9	5	8	18	10	6	16	0	4	10	0	0	10	2
Strip 8		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Nonradioactive—identified by ultraviolet absorption.
† A = femoral artery.
PV = antecubital vein.
HV = hepatic vein.

greatest concentration of activity. The proportion of radioactivity in the more polar portion tended to increase after one hour, however, and that due to cortisol to decrease. Although a part of the spread over the more polar portion of the papers may have been the result of streaking of the cortisol, the findings suggest that there may have been some more polar substance present, particularly in the latter samples. No significant amounts of cortisone could be identified (5).

Area No. 4 of each chromatogram was then eluted, the extracts were pooled, and carrier cortisol (15 mg.) was added. The specific activity of the mixture was 284 counts per minute per milligram (c/min./mg.). Three consecutive recrystallizations of the material from various concentrations of aqueous ethanol were made. The first recrystallization was not counted but the specific activity was 260 c/min./mg. in the second, and 274 c/min./mg. in the third crystalline group. There seemed to be no difference in solubility between the radioactive material and the cortisol added as carrier.

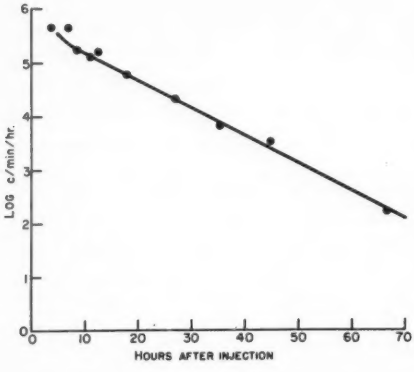

FIG. 2. Total radioactivity in urine as a function of time. Values are expressed as logarithms of counts per minute excreted in one hour.

Within forty-eight hours after administration, 10 per cent of the injected radioactivity was recovered in the feces and 75 per cent was recovered in the urine. The rate of urinary excretion of radioactivity is shown in Figure 2. In this curve the logarithm of the hourly rate of radioactivity excretion during a time-period is plotted as a point midway in the time-period in question. After the first seven hours the excretion rate followed a logarithmic decay curve with a half-life of five and a half hours.

DISCUSSION

The difference in slope of the radioactivity curves among the three sources of blood indicates that the turnover of cortisol in the three was affected by different factors. In comparing the arterial and venous bloods, there is the factor of tissue diffusion. The arterial blood comes into equilibrium with the tissue fluids in the arteriolar and capillary system, and the time involved in diffusion and exchange throughout the available tissue space lengthens the decay period on the venous side of the circulation. It is also understandable that the hepatic venous plasma might show a lower

activity and a more rapid fall than that from the antecubital vein since, against the effect of tissue diffusion, the actual removal of cortisol by the liver and its metabolism to conjugated steroids (6, 7) tends to lower the radioactivity more rapidly.

The one difference which is unexplained by these phenomena is the greater slope and the lower absolute count of the arterial blood in comparison with that of the peripheral and hepatic plasmas. If the plasma in the antecubital vein represents the average blood returning through the peripheral venous circulation, and if no further change occurred in the pulmonary and cardiac circulation, the radioactivity of the arterial plasma should be intermediate between that of the peripheral and that of the hepatic vein. Since this is not the case, it seems probable that either the cerebral, pulmonary or cardiac circulation must involve more rapid turnover than that of the extremities.

Assuming that the first-order portions of the curves represent an approach to equilibrium in the systems measured, the extrapolation of these to zero time would indicate the distribution of the isotope if there had been no removal from the system. On this basis, the arterial blood yielded a value of 21 per cent of the body volume, a close approximation to the extracellular fluid volume. Evidence from analyses of various body fluids, however, indicates that the concentration of 17-hydroxycorticosteroids in these fluids is always lower than that in the plasma unless there is an inflammatory process in the region (8). The distribution in tissues must, therefore, not be limited to the extracellular fluids; certain cells must contain the steroid. The radioautographic studies of Dougherty and Schneebeli (9) indicate that fibroblasts play such a role. Undoubtedly equilibrium with intracellular steroid accounts for the longer half-life of the radioactive steroid in the venous plasma, and for the apparently greater distribution volume (25 per cent) based on this curve.

Although the absolute amounts of 17-hydroxycorticosteroids or of cortisol were not determined in the plasma samples, the test was run so that the samples were drawn during the time when the levels of endogenous 17-hydroxycorticosteroids are most nearly constant (10). On the basis of distribution in 25 per cent of the body mass, and a plasma level of 10 μg. per 100 ml., this 71-Kg. man would have had 1.78 mg. of free cortisol in his body. The added isotopic cortisol (0.42 mg.) would then have momentarily increased the amount by some 23 per cent. This cannot be considered a tracer amount, but it does not seem likely that the metabolism of cortisol could have been seriously affected thereby, in view of the normal range of variation (10).

There is indirect evidence in the human being (7), and direct evidence in the dog (6), that the liver is a major factor in the removal of free cortisol

from the circulation and in its further metabolism. The levels of the free steroid in the hepatic venous blood undoubtedly were lower than in the venous and arterial circulation. In the early samples, before equilibrium with the tissues was complete, it would have been impossible for the hepatic blood to have a specific activity greater than that of the arterial blood. On the basis of this, then, we can place an upper limit on the proportion of free steroid which was removed by one passage through the liver. The activity of the extracts of hepatic plasma per unit plasma volume in the fifteen-minute and thirty-minute samples was 93 per cent of that of the arterial plasma. We can then conclude that not over 7 per cent of the free steroid was removed during one passage. This is considerably lower than that found by Nelson and Harding (6) and probably represents a slower rate of metabolism in man.

The over-all biologic half-life of the isotopic carbon in the subject under study was about five and a half hours. Thus, a mean time of approximately three and a half hours was involved in the metabolism of the steroid and the excretion of its products.

Obviously, this experiment does not supply any information as to the range of variation that can be expected in the population as a whole. It supplements, however, work already published. This can be exemplified by a comparison of a portion of the data presented here with data recently published by Hellman *et al.* (1), who compared the rate of excretion and the metabolic fate of labeled cortisol in 4 human subjects. These workers reported that the urinary excretion curve which they obtained showed a change in slope twenty-four hours after the administration of steroid; our curve showed no significant break during the seventy-two hours in which samples were collected. Since the labeled dose which we injected had five times the activity of the dose used by these workers, the discrepancy may be due to the higher and more consistent counts we were able to obtain in the later urine samples.

SUMMARY

1. 4-C^{14}-17-hydroxycorticosterone was administered to a normal male human subject. It was distributed rapidly until circulating concentrations were equivalent to an apparent distribution volume of 21–25 per cent of the body mass.

2. The half-life of the radioactive compound in the arterial plasma was seventy-two minutes; in the venous plasma, one hundred and fourteen minutes; and in the hepatic plasma, eighty-six minutes.

3. Seventy-five per cent of the injected radioactivity was recovered in the urine, and 10 per cent in the feces within forty-eight hours. On the

basis of urinary excretion of C^{14}, the biologic half-life of cortisol and its metabolic products was approximately five and a half hours.

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ROENTGEN THERAPY IN CUSHING'S SYNDROME WITHOUT ADRENOCORTICAL TUMOR*

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INTRODUCTION

THE purpose of this article is, first to present the results of pituitary irradiation in 12 cases of Cushing's syndrome without detected adrenocortical tumor, and second to analyze the possible reasons for variation in effectiveness of roentgen therapy in these and adequately documented cases noted during a partial review of the literature. Our results, in conjunction with those of others, suggest that the effectiveness of a given total dose of x-ray to the pituitary is greater if the length of the course in days is relatively short.

METHODS AND MATERIALS

The records of the Medical Service, the Endocrine Clinic, Section of Medicine, and the Department of Radiology of the Hospital of the University of Pennsylvania, and the private records of two of us were reviewed for the period 1932–1952. Only those patients were included in this study who fulfilled the diagnostic requirements of Cushing's syndrome without demonstrable adrenocortical tumor, and who were treated by roentgen therapy of the pituitary region. Factors in the diagnosis of Cushing's syndrome are indicated in the case histories (Appendix), as are the methods used in the attempt to eliminate the possibility of adrenocortical tumors. The inclusion of Case #5 (M.H.) requires comment. The left adrenal gland had been removed from this patient nine weeks prior to instituting roentgen therapy. There was no evidence of improvement until the usual time after completion of roentgen therapy. We have included this patient, in view of this and other considerations discussed in the case history (*see* Appendix).

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The urinary total neutral 17-ketosteroids were determined by a modification of the method of Holtorff and Koch (1) and the "corticoids" as neutral lipid-soluble reducing material (2) after acid hydrolysis and chloroform extraction for one hour.

Roentgen therapy technics: Various irradiation technics were used in the 12 cases reported (Table 1). In general, multiple cross-firing portals were employed. Treatment was given five days a week, excluding holidays, in most instances. All doses are stated in "tissue roentgens" at the center of the pituitary fossa. This dose is calculated from the known amount of radiation delivered at the surface of the head by appropriate subtractions for absorption and additions for backscattering. These calculations are subject to several significant sources of error, including the assumption that portal localization was perfect, inaccurate measurement of the distance from the portal surface to the pituitary, bone absorption, and curvature of the head's surface. Most of the possible errors tend to make the computed dose higher than the actual dose. It seems unlikely that our reported doses are more than 25 per cent greater than the doses the pituitary region actually received. Except in Cases 7 and 11, and the first two courses in Case 12, all radiation therapy was given in the Department of Radiology, Hospital of the University of Pennsylvania.

The term "tissue roentgens per day" indicates the average daily dose, obtained by dividing the total dose in "tissue roentgens" by the total number of days over which the course of the therapy extended (thus including some days on which therapy was not given).

RESULTS

Part I. *Response to roentgen therapy in the Hospital of the University of Pennsylvania series*

A. *The response to total dose of irradiation (all courses).* Our experience with roentgen therapy to the region of the pituitary in 12 patients with Cushing's syndrome (without demonstrated adrenocortical tumor) is summarized in Table 1. The subjects are listed in order of decreasing "tissue roentgens per day" (first course). Results were excellent in 5 patients, fair in 2, and poor in 5 (from the *initial* course of therapy).[1] In 1 (E.R.) of the latter 5, after three courses failed, the effect of a fourth course of therapy was rated fair. Results were judged to be fair on the basis of some im-

[1] *Note added in proof.* Investigation in the summer of 1956 indicated that all 5 of the patients with excellent results (Cases 1 to 5) continue without evidence of relapse, from five and a half to over seven years after the initial course of roentgen therapy. One patient (Case 7) continues to have only a fair response; the evidence of Cushing's syndrome is such that additional roentgen therapy is being considered.

TABLE 1. RESULTS OF ROENTGEN THERAPY TO THE PITUITARY IN CUSHING'S SYNDROME WITHOUT ADRENOCORTICAL TUMOR—HOSPITAL OF UNIVERSITY OF PENNSYLVANIA SERIES.

Case No.	Patient			Duration (mos.)		Date 1st x-ray	Dose of irrad. to pit. region			Cushing's stigmata§		Blood pressure (mm. Hg)		Impairment glucose metab.		Body weight (lbs.)		Menses		Remarks
	Name	Age (yrs.)	Sex	Onset*	Follow-up**		Tiss. r per day†	Tot. dose (tiss. r)	Duration‡ (days)	Before	After	Before	Last	Before	After	Before	Last	Before, absent (mos.)	After, ret'd. (mos.)	
1	D.W.	31	F	9	41	Dec. 27, '49	129	4500	35	+++	0	145/100	124/80	++	0	115	113	9	8	Excellent
2	V.S.	22	F	60	36	Feb. 3, '51	126	3900	31	+++	0	130/112	108/88	±	±	162	124	7	7	Excellent
3	A.L.	32	F	36	61	Mar. 12, '49	112	3800	34	+++++	0	170/110	128/78	++	0	160	139	36	6	Excellent
4	E.G.	17	M	48	43	June 2, '50	105	4300	41	+++	0	190/100	148/80	+	0	260	223	—	—	Excellent
5	M.H.	24	F	16	51	Nov. 28, '49	101	5250	52	++++	0	158/110	132/92	++	0	189	152	9	1	Excellent Lt. adrenalectomy 2 mo. prior to initial x-ray treatment
6	A.C.	42	F	6	4	June 4, '37	77	614	8	++++	+++	120–146 80–88	150/90	++	−	128	129	1	0	Poor
					approx. 36	Oct. 12, '37	56	1016	18	+++	Unknown	130–190 80–130	170/110	−−	−−	129	−−−	0	0	Poor Died in 1940; cellulitis after several strokes; no autopsy
7	S.M.	19	M	36	6	June 9, '52	71	1570	22	+++++	+++	180/130	160/130	++	+	145	138	—	—	Fair
					20	Dec. 8, '52	64	1470	23	+++	++	160/130	150/110	+	0	138	130	—	—	Fair
8	B.B.	39	F	48	4	Aug. 23, '48	67	1140	17	++++	+++	170/100	170/108	++	++	149	155	?3	1	Fair
					26	Jan. 3, '49	76	1140	15	+++	+++	No change		++	++	155	168	Fairly regular		Fair
					59	Oct. 20, '50	118	3900	33	+++	+++	No change		++	+	168	169	Fairly regular		Fair Died from cerebral hem., July 6, '53
9	E.R.	21	M	60	2	July 2, '32	67	400	6	+++++	+++++	150/118	140/120	++	?	185	189	—	—	Poor
					4	Aug. 18, '32	160	800	5	+++++	+++++	140/120	150/110	−−	−−	189	186	—	—	Poor
					12	Nov. 14, '32	68	1836	27	+++++	++++	150/110	180/110	−−	−−	186	181	—	—	Poor
					88	June 22, '53	138	965	7	++++	+	180/110	140/120	−−	−−	181	167	—	—	Fair Died from cerebral hem., Nov. 10, '39
10	F.G.	39	F	18	54	July 6, '37	55	600	11	+++	+++	170/100	180/100	++	++			12	3 (long intervals)	Poor Subsequent course unknown
11	R.S.	45	F	24	6	May 10, '51	54	4550	85	+++++	+++++	190/120	190/120	++++	++++	140	126	2	0	Poor Died from pulm. edema, Nov. 6, '51
12	G.S.	28	F	24	24	Oct. 14, '39	10	500	52	++++	+++++	180/110	180/110	+++	++++	120	−−−	17	0	Poor
					36	1941	?	540	?	+++++	++++	Essentially no effect		++	++			29	0	Poor Exploration of adrenals, May 28, '41
					111	Apr. 12, '42	74	886	12	++++	+++			+++	++	No significant change		47	50	Poor
					117	Jan. 7, '49	89	2570	29	++++	++++			−−	−−			Scant at intervals		Poor Died from cerebral hem., July 19, '49

* Duration of symptoms suggestive of Cushing's syndrome prior to initial x-ray treatment.
** Period of observation after initial x-ray treatment.
† Total tissue r/duration of therapy course in days.
‡ Total period over which irradiation was administered, including first and last days.
§ Rough estimate of general severity of symptoms, including hirsutism, acne, purple striae, ecchymosis, and round face with ruddy complexion; graded 0 to +++++.

provement, but continuation of hypertension and other signs of Cushing's syndrome to some degree. Abstracts of case histories are listed in the Appendix and the clinical courses of 4 of the 5 patients having excellent results and 2 of the others are charted in Figures 1 to 6. Photographs of the patients before and after treatment accompany the charts.

Analysis of the data concerning the relative severity of the syndrome and age of the patients indicated that there was no significant difference between the group of 5 with excellent results and the group of 7 with fair or poor results. The estimate of mean duration of the syndrome prior to initial x-ray therapy was essentially the same for both groups (three years).

Response to initial course of irradiation. The total tissue dose per course and average "tissue roentgens per day" are shown in Table 1. The total dose for the 5 patients who showed an excellent response was high (mean 4350; range 3800–5250 tissue r). With 1 exception, the total dose for the 7 patients with a poor or fair response was lower (mean 1339; range 400–4550 tissue r). The average "tissue roentgens per day" for the initial course ranged from 101 to 129 roentgens (mean 113) for the 5 with excellent response, and ranged from 10 to 85 roentgens (mean 62) for the 7 with fair and poor results. The relationship of average "tissue roentgens per day" to therapeutic response is considered in Part II.

Response to subsequent irradiation therapy. Six of the 7 patients not showing an excellent response to the initial course were subjected to one or more subsequent courses of irradiation—with little effect. One patient (E.R.) apparently had a fair response after his fourth course of irradiation (965 tissue r given at the mean rate of 138 r per day; Fig. 6).

B. *Time interval to appearance of marked improvement following initial roentgen therapy.* Table 2 shows the variability in the approximate time of appearance of marked improvement in some of the signs and symptoms of Cushing's syndrome. In general, six months elapsed before marked improvement was manifest in the 5 patients with excellent results. However, all 5 showed appreciable changes within three months.

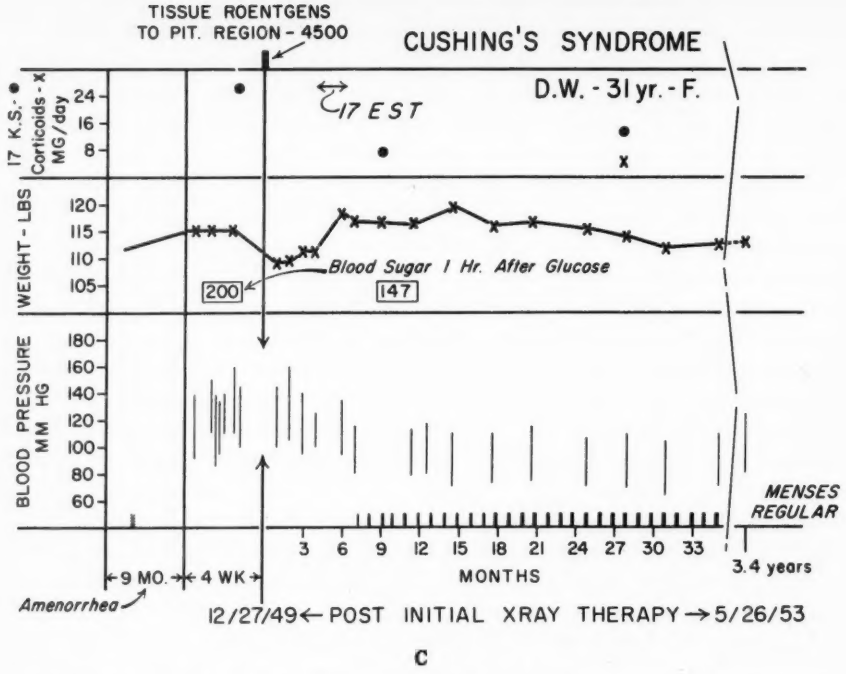
Comment: Others have also noted the delay after roentgen therapy in the appearance of marked improvement (3–6). The period until this is achieved appears to be considerably greater than the two or three months required for essentially complete remission following subtotal or total adrenalectomy (7).

C. *Complications of irradiation therapy:* Three major complications have been reported: 1) hypophyseal insufficiency, 2) necrosis of brain or other tissues traversed by the radiation, and 3) sudden, unexplained death during or just following the treatment period.

Hypophyseal insufficiency was apparently experienced by 1 of our patients (A.L., Case 3) who received a total dose of 3800 tissue r (average

† Total tissue r/duration of therapy course in days.
‡ Total period over which irradiation was administered, including first and last days.
§ Rough estimate of general severity of symptoms, including hirsutism, acne, purple striae, ecchymosis, and round face with ruddy complexion; graded 0 to +++++.

A B



C

FIG. 1. *Case 1, Subject D.W.* **A.** Before treatment. **B.** Twenty-one months after initiation of x-ray therapy. **C.** Chart of the course. The result is classified as excellent.

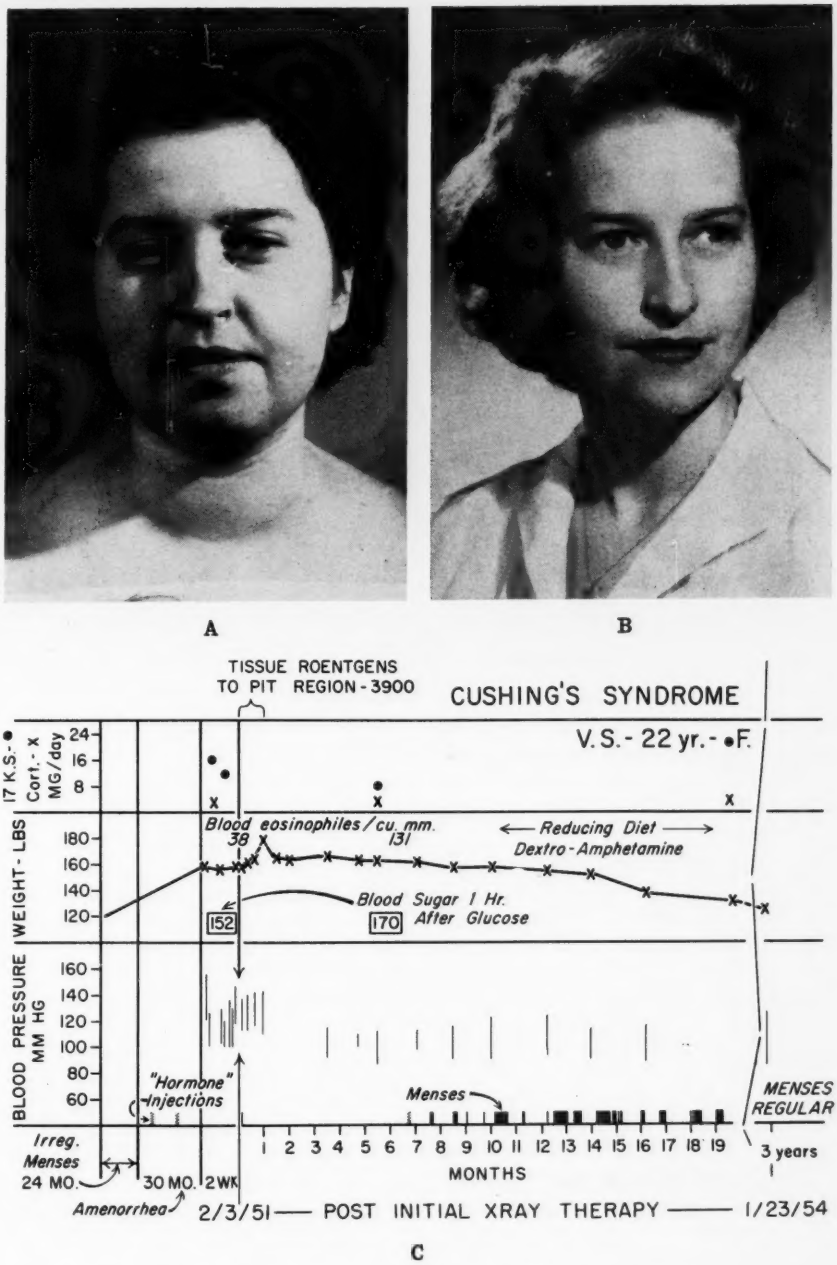


FIG. 2. *Case 2, Subject V.S.* **A.** Before treatment. **B.** Twenty-four months after initiation of x-ray therapy. **C.** Chart of the course. The result is classified as excellent.

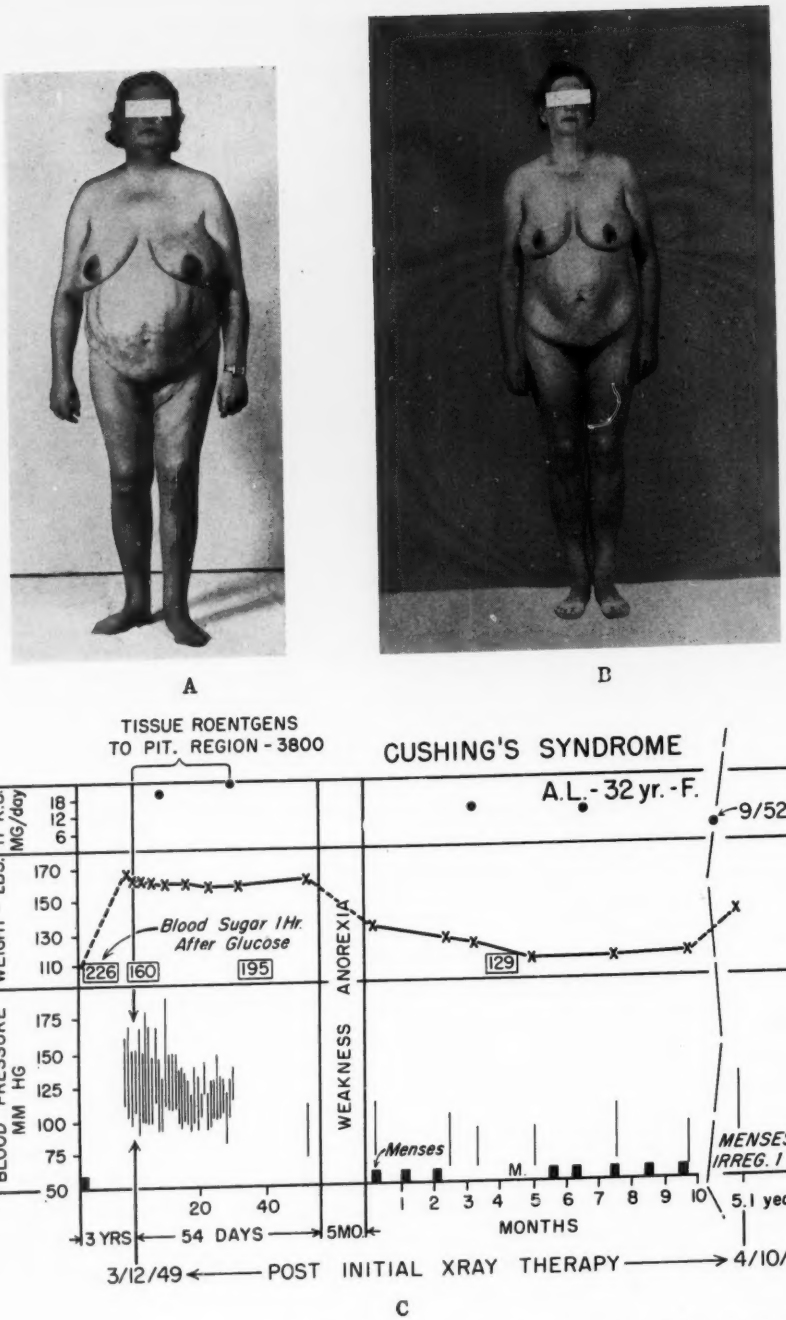
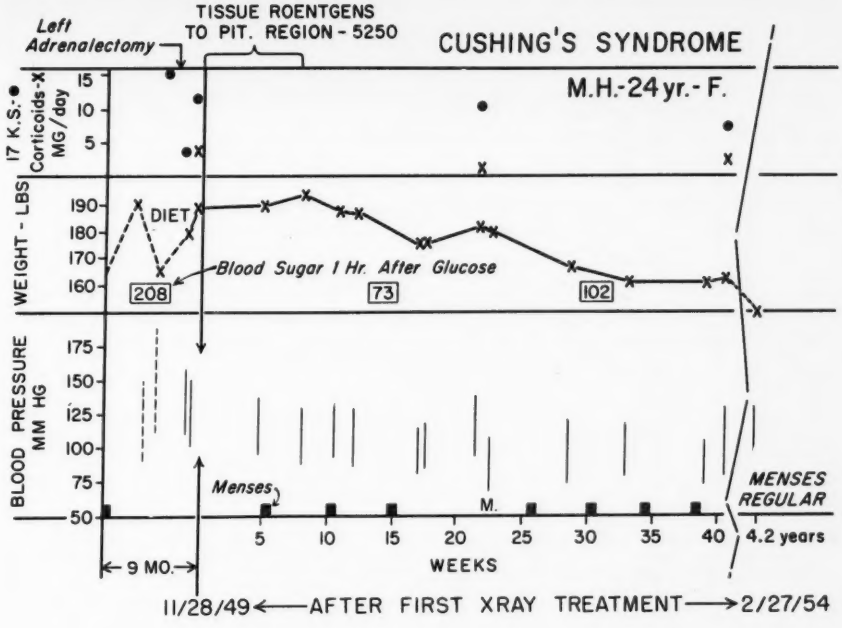


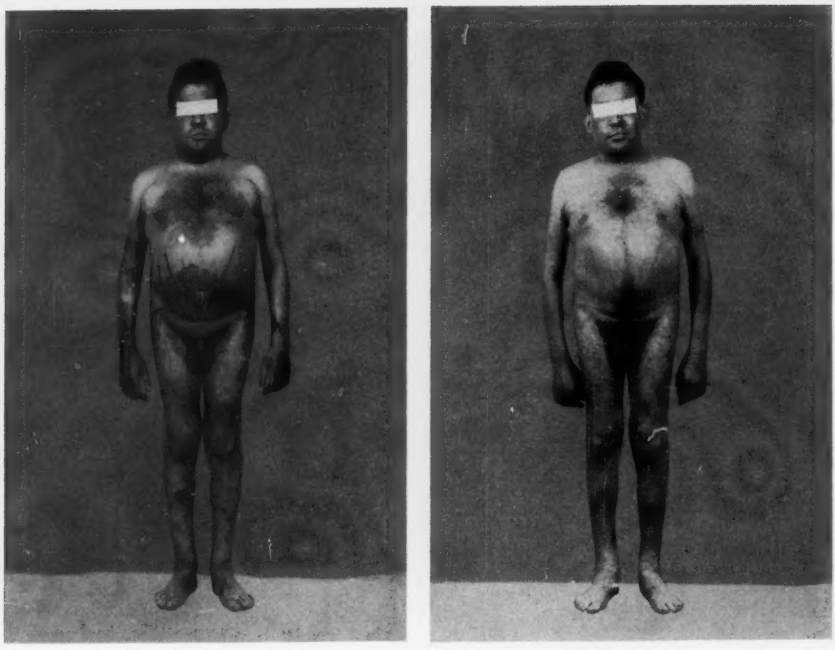
FIG. 3. *Case 3, Subject A.L.* **A.** Before treatment. **B.** Thirty-one months after initiation of x-ray therapy. **C.** Chart of the course. The result is classified as excellent.

A B



C

FIG. 4. *Case 5, Subject M.H.* **A.** Before treatment, two months after left adrenalectomy. **B.** Forty-two months after initiation of x-ray therapy. **C.** Chart of the course. The result is classified as excellent. Removal of the left adrenal had essentially no effect upon the clinical picture of Cushing's syndrome (*see* Appendix).



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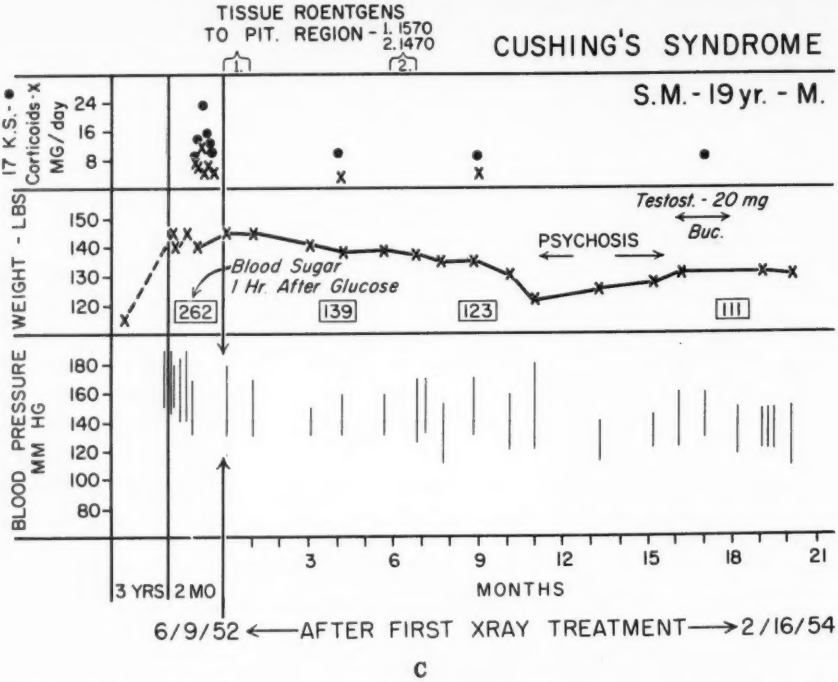

C

FIG. 5. *Case 7, Subject S.M.* **A.** Before treatment. (The widespread skin lesion on lower abdomen and arms is miliaria rubra). **B.** Fourteen months after initial x-ray therapy and eight months after second course. Hypertension continued. **C.** Chart of the course. The result is classified as fair.

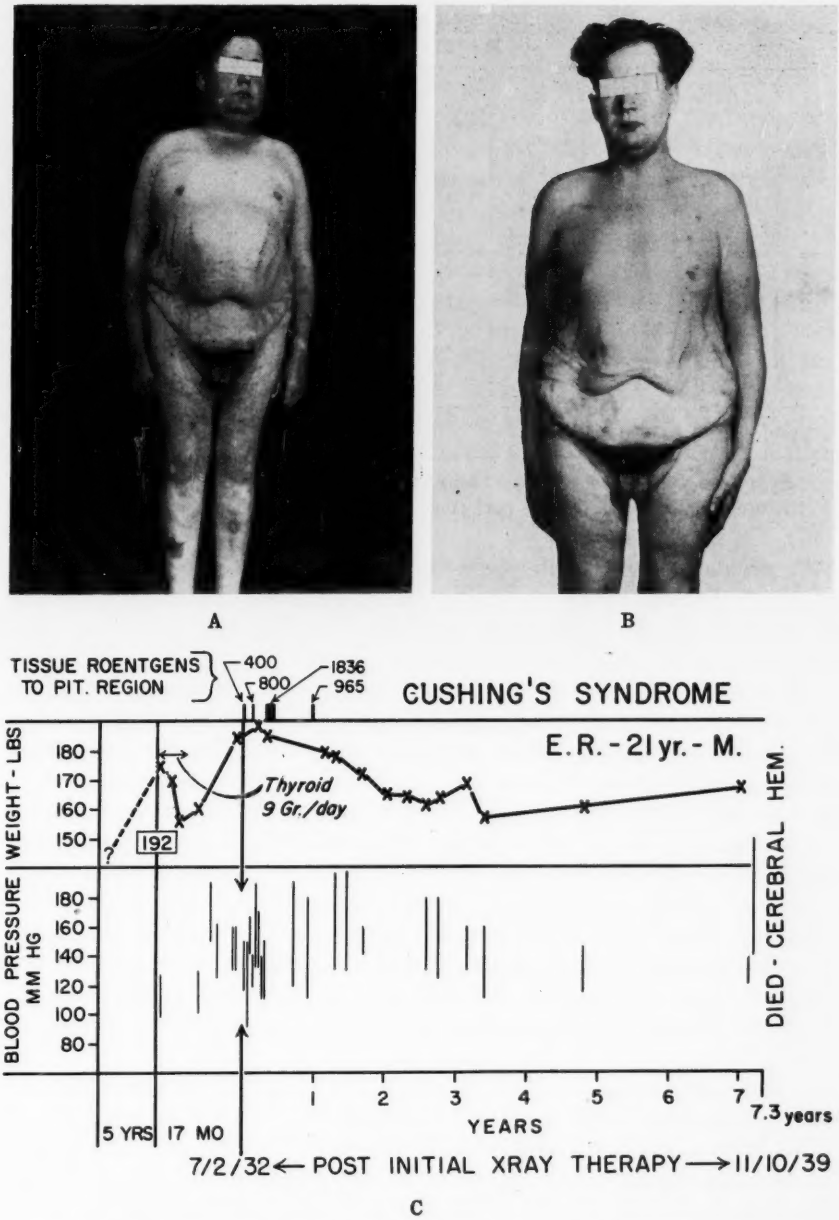

FIG. 6. *Case 9, Subject E.R.* **A.** Before treatment. No change in appearance until after fourth course of irradiation. **B.** Forty-six months after fourth course of x-ray therapy. The patient felt considerably better but still had hypertension and died of a cerebral hemorrhage two and a half years after this picture was made. **C.** Chart of the course. The result is classified as fair.

TABLE 2. TIME REQUIRED FROM FIRST DAY OF IRRADIATION UNTIL MARKED IMPROVEMENT (MONTHS)

Case No.	Patient	Fall of diastolic B.P. to <85 mm. Hg	Return of menses	Marked improvement in				
				Psyche	Facial and body contour	Acne	Purple striae	Glucose tolerance†
1	D.W.	7	8	6	6	6	*	10
2	V.S.	6	7	10	12	4	10	*
3	A.L.	2	6	7	4	*	18	11
4	E.G.	2	male	2	4	*	4	9
5	M.H.	5	1	3	9	10	12	3
Average		4	6	6	7	7	11	—

* Symptom or sign not present, or only slight, before irradiation of pituitary region.
† Glucose tolerance tests were performed at infrequent intervals.

112 r per day). Unfortunately, she was not observed by us during the five-month period when this occurred (Fig. 2c), but she stated that she lost 30 pounds in weight, suffered from anorexia and weakness, and lost most of the hair on her face, arms and legs.

Comment: Hypophyseal insufficiency in a patient treated for Cushing's syndrome by pituitary irradiation has also been reported by Weller (8). Spillane (9) reported optic and cortical atrophy beginning two years after a dose of 6000 tissue r delivered in thirty-two days (188 tissue r per day) to a patient with Cushing's syndrome, and briefly reviews several similar cases. Delayed necrosis of the brain following irradiation for other diseases has been the subject of several reports. In a recent communication, Malamud *et al.* (10) re-examined these reports and commented upon the divergent opinions concerning pathogenesis. Boden (11) calculated the radiation tolerance of the brain stem on the basis of his own experience plus a review of reported cases of radiation necrosis. The line representing his suggested tolerance dose for various treatment periods is reproduced in Figure 7.

Sudden death shortly after radiation therapy of Cushing's syndrome has been reported by Kerr (12) and by Sosman (3). Kerr's patient received 2157 tissue r in fifteen days and experienced one week of improvement followed by sudden death. Sosman's patient (his Case III) was given approximately 500 tissue r over four days and died suddenly at home three weeks later without having shown improvement. The predilection of

Cushing's syndrome patients to vascular disease must be considered in weighing the role of roentgen therapy in these deaths.

D. *Cause of death:* Five of our 7 patients showing a fair or poor response have died; 1 cannot be traced (F.G.) and the other (S.M.) is living but exhibits hypertension and other signs of adrenocortical hyperactivity (*see* Appendix). All 5 of those who died had cerebrovascular accidents; in 3 (B.B., G.S. and E.R.) it was the immediate cause of death. In A.C., the primary cause of death was "cellulitis" and R.S. succumbed during an attack of pulmonary edema. Survival among the 5 who died averaged five years after initial roentgen therapy (range, 6 to 117 months).

Comment: Plotz *et al.* (5) reviewed the autopsy findings in their series and in 107 autopsies reported in the literature. They reported that cardiovascular complications were the immediate cause of death in 40 per cent of the young, predominantly female adults in their series. However, bacterial infection was the cause of 46.6 per cent of the deaths (presumably because most of the reported patients died before the advent of antibiotic therapy).

Part II. *Dose and duration of roentgen therapy in relation to therapeutic effect—Published cases plus cases at the Hospital of the University of Pennsylvania*

The dependence of the biologic effect upon the dose and the duration of roentgen therapy has been established for some diseases and certain biologic test objects (13). We have analyzed our own 12 cases and 24 of the cases in the literature (3, 5, 6, 9, 14, 15) from this point of view.

For the purposes of this analysis, cases were classified as improved only if *marked* improvement had occurred within one year of the *initial* course of irradiation and previous to subsequent pituitary irradiation or adrenal surgery. Thus, cases classified in Table 1 as having excellent results are considered as improved; those showing only fair or poor results are classified as unimproved. The possible effect of occasional treatment with desiccated thyroid, steroid hormones, electroshock therapy or irradiation of the parathyroid or adrenal region cannot be evaluated. The few cases in which such therapy was given have been included because there is little evidence of the effectiveness of these procedures.

The majority of reports reviewed were not suitable for this analysis. Cases are omitted from Table 3 because of: 1) insufficient data for determination of the approximate total tissue dose and total duration of therapy and the approximate time and extent of improvement; 2) discovery of an adrenocortical tumor at operation or autopsy; 3) adrenalectomy or death within approximately six months after the first day of irradiation; 4)

fleeting improvement, then relapse within six months of the first day of irradiation, and 5) marked improvement with multiple courses within one year, unless the marked improvement was clear-cut before the second course. (The cases with multiple courses of roentgen therapy failing to respond within one year of the initial course were included, since it seems improbable that the subsequent course would inhibit any possible effect of the first course.)

Analysis of results. The effects of irradiation (initial courses) are shown in Table 3, listed in order of decreasing "tissue roentgens per day." The 12 cases from the Hospital of the University of Pennsylvania are included. Upon inspection of the table it may be seen that the effectiveness of therapy appears to be related to the "tissue roentgens per day," and not to total dose. The chi square test indicated a highly significant correlation ($P = <0.001$) between the results and tissue dose of roentgens per day, independent of total duration. There was no significant correlation ($P = 0.3$) between total dose and results.

DuSault (13) has recently reviewed the evidence indicating the interdependence of radiation dose and duration in producing biologic effects. In general, the longer the total period over which the irradiation is administered, the greater the total dose needed to produce the biologic effect. If the course of irradiation consists of approximately one treatment per day for one to several weeks, the relationship generally follows a straight line when the logarithm of total dose is plotted against the logarithm of the elapsed treatment time in days after the first day of treatment. The data listed in Table 3 are plotted in this manner in Figure 7. In this figure, 14 of the 18 improved cases lie on or above a straight line which can be fitted by eye; 16 of the 18 unimproved cases are below this line. Thus, unless the data are biased, this line represents the approximate minimum dose necessary in any given period of irradiation (within the limits charted) to produce remission in most cases.

The calculation "tissue roentgens per day," as used in Table 3 and elsewhere in this paper, furnishes a first approximation of the position of any case with respect to this minimum effective time-dose line. However, since the slope of the line is not 1.0, but 0.75, the minimum effective dose per day changes with time. For example, cases lying exactly on the line would have an average r/day of 110 if treatment were completed in four days, 96 r/day if treatment required fourteen days, or 77 r/day in forty days.

Comment: These results suggest that, at least for treatment periods ranging from four to fifty days, the "average tissue roentgen per day" is a very important element in determining the result. Since the effect of radiation appears to be less cumulative, the irregularity of treatment fractionation due to such factors as holidays or intercurrent illness would have

TABLE 3. CUSHING'S SYNDROME: EFFECT OF INITIAL COURSE OF IRRADIATION TO THE PITUITARY REGION

Reference	Patient			X-ray therapy			Results†	Improved
	Name	Age (yrs.)	Sex	Course (days)	Total dose total r	Tissue r per day*		Total
Spillane (9)	M.C.	26	M	4	1700	425	0	
Spillane (9)	G.K.	26	M	32	6000	188	+	
Sosman (3)	E.F.	30	M	4	640	160	+	5/6
Spillane (9)	J.T.	23	M	19	3000	158	+	
Table 1 (this study)	D.W.	31	F	35	4500	129	+	
Table 1 (this study)	V.S.	22	F	31	3900	126	+	
Luft (6)	7‡	16	M	6	750	125	0	
Sosman (3)	L.D.	37	F	17	2000	118	+	
Luft (6)	3	45	F	8	900	113	+	5/6
Jamin (14)		20	M	4	450	113	+	
Table 1 (this study)	A.L.	32	F	34	3800	112	+	
Luft (6)	1‡	16	F	7	750	107	+	
Table 1 (this study)	E.G.	17	M	41	4300	105	+	
Table 1 (this study)	M.H.	24	F	52	5250	101	+	
Sosman (3)	W.S.	27	M	9	900	100	0	4.5/6§
Plotz (5)	C.T.	17	M	23	2300	100	+	
Davidoff (15)	L.S.	30	M	15	1440	96	+	
Luft (6)	2‡	23	F	8	750	94	+	
Luft (6)	4‡	41	F	8	750	94	0	
Plotz (5)	A.D.‡	27	F	12	1100	92	0	
Plotz (5)	J.T.‡	24	F	13	1200	92	0	1.5/6§
Plotz (5)	R.C.	30	F	16	1400	88	0	
Luft (6)	8	16	F	8	650	81	+	
Plotz (5)	R.K.	28	F	33	2550	77	0	
Table 1 (this study)	A.C.‡	42	F	8	614	77	0	
Plotz (5)	R.W.	33	M	18	1300	72	+	
Table 1 (this study)	S.M.†	19	M	22	1570	71	0	
Plotz (5)	B.C.‡	31	F	14	950	68	0	1/6
Table 1 (this study)	E.R.‡	21	M	6	400	67	0	
Table 1 (this study)	B.B.‡	39	F	17	1140	67	0	
Plota (5)	MdeT.	46	F	36	2275	63	0	
Table 1 (this study)	F.G.	39	F	11	600	55	0	
Table 1 (this study)	R.S.	45	F	85	4550	54	0	1/6
Plotz (5)	S.G.	29	M	25	1200	48	0	
Plotz (5)	M.F.	17	F	31	1200	39	+	
Table 1 (this study)	G.S.‡	28	F	52	500	10	0	

* Tissue r per day $= \frac{\text{Total dose tissue roentgens}}{\text{Total course of roentgen therapy in days}}$

† 0 = Only fair or no improvement; + = marked improvement (*see* text).

‡ A second course was given within one year (*see* text for discussion of rationale of inclusion of these cases).

§ Luft Cases 2 and 4 apparently received irradiation at the same rate per day. In accordance with accepted statistical practice ½ value for each observation has been assigned to each group of 6.

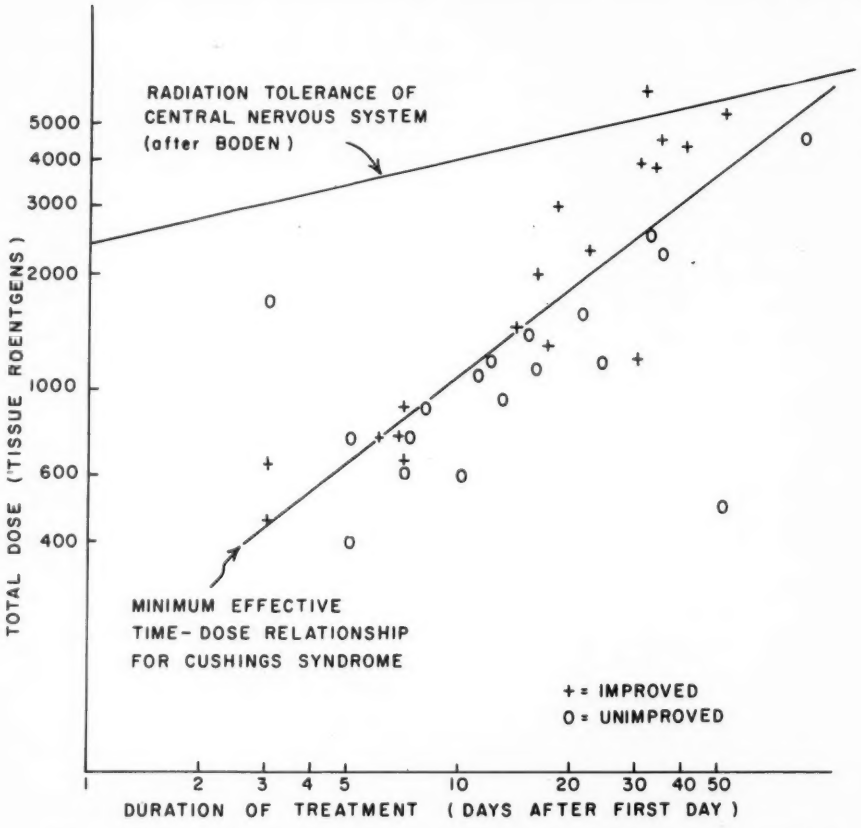

FIG. 7. Relationship of total radiation delivered to pituitary region and length of course to improvement of Cushing's syndrome. The data listed in Table 3 are plotted on a log-log scale according to the method of Strandquist and others (13). The minimum effective time-dose line has been fitted by inspection. Fourteen of the 18 improved cases lie on or above this line and 16 of the 18 unimproved cases are below. (Chi square = 26.7; $P < 0.01$). The upper line indicates Boden's estimate of radiation tolerance of the central nervous system (11). In the 1 case lying above this line, cortical atrophy was reported to have developed (9).

a greater influence than that noted in cancer therapy. The relatively small cumulative effect is indicated by the slope of the minimum effective time-dose line in Figure 7. This is about 0.75, whereas the slope of such lines for the treatment of various malignant conditions (13) ranges from 0.2 to 0.4 Such a slope suggests a relatively rapid recovery rate of the cells of the hypothalamus or pituitary, which presumably are functioning ab-

normally in Cushing's syndrome without adrenocortical tumor. From the practical point of view, it indicates the possibility of obtaining good results with relatively low total doses if the irradiation be given daily over a short period of time (*i.e.*, at a high "tissue roentgen per day" level).

DISCUSSION

Despite the "statistical significance" of these results, we feel that no firm conclusion can be drawn at this time with respect to the most effective technic for roentgen treatment in Cushing's syndrome. The cases are drawn from different series, and we have no assurance that all pertinent details are included in this analysis. Accordingly, it is possible that some unrecognized variable accounts for the apparent correlation between average daily doses and good results.

However, we are convinced that careful studies are justified to test the hypothesis that Cushing's syndrome will respond to a relatively low total roentgen dose delivered at a high average daily increment (for instance, 1000 roentgens in five consecutive days or 1500 tissue roentgens in ten days with only one holiday from treatment). The safety of these doses (Fig. 7) is suggested by the report of Boden (11), although he presented few data covering treatment courses of less than seventeen days' duration. Arnold's (16) recent observations in monkeys suggested that the therapeutic benefits following irradiation of the pituitary region are due to effects on the hypothalamus rather than the pituitary. There is considerable evidence indicating a hypothalamic influence upon the release of corticotropin. It, therefore, seems logical that the size of the x-ray portal should be large enough to include the hypothalamus, as has usually been the case in the past. It seems appropriate to emphasize the necessity for complete specification of roentgen-therapy technic in future reports, so that the experience of many centers may form a more adequate basis for evaluation of roentgen therapy.

Prior to starting irradiation treatment for Cushing's syndrome, efforts should be made to detect a tumor by means of body-section films and intravenous urography (possibly with presacral gas-injection studies (17)); hormonal assays (18); and determination of the effect of cortisone or hydrocortisone upon 17-ketosteroid excretion (19). After irradiation, if there is progression of the disease, or no clear signs of remission within three to six months, a total or subtotal adrenalectomy should be performed. It is well recognized that only surgical exploration can completely rule out the presence of an adrenocortical tumor. Sprague (18) has recently reviewed the results of adrenalectomy in a large series of patients having Cushing's syndrome with and without adrenocortical tumor; hyperplasia was five times as common as tumor in this series.

SUMMARY

During the period 1932–1952, roentgen irradiation to the pituitary region was used in the treatment of 12 patients with Cushing's syndrome without demonstrated adrenocortical tumor. In 5 cases there was "complete" remission; some signs of remission were usually evident within three months but improvement was not well established until about six months following therapy. In the other 7 cases the response was unsatisfactory, although in 3 there was partial remission with continuation of high blood pressure and other signs.

A partial review was made of the published cases of Cushing's syndrome without detected adrenocortical tumor; 24 were found suitable for evaluation. In these, together with our 12 cases, effectiveness of roentgen therapy was better correlated with the mean dose of tissue roentgens delivered per day of total course than with either total dose or duration of roentgen treatment. The estimated total tissue dose has been plotted against duration of the roentgen therapy in days, on log-log paper. A line may be drawn which separates, in a highly significant fashion, the cases which responded from those which did not.

It is suggested that careful clinical trials be made to test the hypothesis that Cushing's syndrome not associated with adrenocortical tumor will respond to a relatively low total dose if delivered as a relatively high daily increment.

Acknowledgments

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APPENDIX

Case **1.** *D.W., white, female*

This 31-year-old housewife was admitted to the Medical Service of the Hospital of the University of Pennsylvania on December 2, 1949. She had been amenorrheic for nine months, and had an eighteen-month history of increasing acne, insomnia, indigestion, loss of libido, nervousness, depression, easy fatigability, and puffiness of her face and hands. An increased growth of hair on her body and cheeks, mental confusion, poor memory and headaches had been noted. Her course is charted in Fig. 1.

A blood pressure of 145/100 mm. Hg was representative of many varying values. On physical examination, the following signs were observed: prominence of the eyes, puffiness of the face with large jaws, a thick neck, a "buffalo hump" over the upper dorsal spine, mild hirsutism of the face and arms, acne and seborrheic dermatitis of the face and back. She had no purple striae.

The hemoglobin reading was 77 per cent, basal metabolic rate −20 per cent, and serum cholesterol level 420 mg. per 100 ml. Glucose tolerance was impaired; fasting, 30-minute, 1-hour and 2-hour blood sugar values were 78, 146, 200 and 169 mg. per 100 ml., respectively. On x-ray examination, the skull was normal and there was no evidence of adrenocortical tumor.

Treatment consisted of small doses of insulin, 30 mg. of desiccated thyroid daily, and pituitary irradiation totalling 4500 tissue roentgens over a 35-day period starting December 27, 1949.

Four months later she had not improved, was profoundly disturbed emotionally, lacked decision, had insomnia and depression and was worried about her frigidity. At this time her only medication was desiccated thyroid. She was given 17 electroshock treatments at the Philadelphia Psychiatric Hospital (under the care of Dr. Samuel Cohen) between May 1, 1950 and July 6, 1950. Following this, she felt markedly improved. The blood pressure dropped to 116/80 mm.Hg and has remained at normal levels. Menses returned on August 4, 1950, and have been regular since then. In October 1950, a glucose tolerance test was performed; the fasting, 30-minute, 1-hour, 2-hour and 3-hour blood sugar values were 68, 119, 147, 101 and 87 mg. per 100 ml., respectively. Neutral 17-ketosteroid excretion, which was 27.3 mg. per twenty-four hours before pituitary irradiation, returned to normal levels. Psoriasis, which had disappeared with the onset of Cushing's syndrome, reappeared in December 1950. Three years and four months after pituitary irradiation and three years after electroshock treatment, she was "well" and the result has been classified as excellent.

Case 2. V.S., white, female

This 22-year-old telephone operator was first admitted to the Hospital of the University of Pennsylvania on January 27, 1951, complaining of obesity, headaches and menstrual abnormality. She had developed normally until the age of 16 years when, following an attack of "pneumonia," she noted a marked increase in her appetite and a gain in weight from her usual 120 pounds to 159 pounds in a period of six months. From the age of 17 years, her periods had been scanty and irregular. When first seen, she had not had a period without "an injection" for well over a year. At the age of 19, severe acne of the face, back and upper chest appeared; she had not had acne during adolescence. A "buffalo hump" also developed over the upper dorsal and lower cervical vertebrae, as well as distention and purple striation of the abdomen. Migraine had occurred about once a week during the previous year. There had also been a slight increase in facial hair and slight loss of hair from the scalp. During this five-year period, she had become moody and seclusive.

Physical examination showed only moderate acne and some increase in hair on her rounded, but not particularly plethoric face. There were two large bruises on the right leg. The blood pressure before treatment varied; diastolic pressures of 112 to 120 mm.Hg were frequent.

Roentgen studies of the skull, chest and urinary tract yielded essentially negative findings. There was no evidence of adrenal tumor by body section films. Glucose tolerance was slightly impaired, the two-hour blood sugar value being 125 mg. per 100 ml. The eosinophil count was low (38 per cu.mm.), the hemoglobin reading was 105 per cent, the red blood cell count was 5.1 million and the white cell count 8.5 thousand per cu. mm., with 82 per cent neutrophils and 18 per cent lymphocytes. The basal metabolic rate was −24 per cent. Kolmer and Kline tests yielded negative results. Values for blood urea nitrogen, and serum sodium, potassium, calcium, phosphorus and alkaline phosphatase levels were within normal limits, except that one of three calcium values was elevated (12.6 mg. per 100 ml.). Urinary excretion of gonadotropins was normal. Urinary 17-ketosteroid excretion was 12 mg. per twenty-four hours, and corticoid excretion 2.7 mg. per twenty-four hours—both within normal limits.

A total dose of approximately 3900 tissue roentgens was delivered to the pituitary region over thirty-one days, beginning on February 3, 1951.

Her course is shown in Figure 2. By May 19, the blood pressure was 114/92. The acne disappeared, purple striae gradually became less marked and appeared as "silver"

rather than purple. Weight loss was probably aided by dextro-amphetamine and some voluntary restriction of food intake. When last seen, approximately thirty-six months after x-ray therapy, she had lost a total of 54 pounds (Fig. 2), blood pressure was normal, migraine attacks were unusual and her personality had become more "outgoing." Her menstrual periods, previously somewhat irregular had been regular for five months. The result is classified as excellent.

Case 3. A.L., white, female

This 32-year-old white housewife was referred to the Hospital of the University of Pennsylvania in 1949 by Dr. Herman Gold, with a diagnosis of Cushing's syndrome made on the basis of a three-year history of a 55-pound weight gain, amenorrhea, purple-red striation of the skin of the abdomen, neck, flanks and buttocks, purplish discolorations of her face and neck, moon face, acne, mild hirsutism, and extreme weakness, nervousness and irritability.

The blood pressure was 170/110 mm.Hg. In peripheral blood the absolute eosinophil count was 38 per cu. mm., and the proportion of lymphocytes was 15 to 20 per cent. There was impaired glucose tolerance, with fasting, 30-minute, 1-hour and 2-hour blood sugar values of 87, 133, 195 and 286 mg. per 100 ml., respectively. The levels of serum sodium, potassium, chloride, calcium, phosphorus and protein were within normal limits. X-ray examination revealed no evidence of suprarenal, intrathoracic or pituitary tumor.

She was given 3800 tissue roentgens to the pituitary gland over a 34-day period, beginning March 12, 1949. In Figure 3 are shown the course of the disease and the appearance of the patient before and after treatment.

During a five-month period when she failed to return to the clinics, there was a rapid weight loss of 30 pounds and symptoms suggestive of Simmonds' disease. At the end of this time, her menses returned; except during a pregnancy which ended with a miscarriage, they were regular.

Ten months after irradiation therapy, her status was re-evaluated. The blood pressure was 90/80 mm.Hg and there was a marked decrease in the degree of hirsutism and acne. Improved glucose tolerance was demonstrated by fasting, 30-minute, 1-hour and 2-hour blood sugar values of 87, 117, 129 and 108 mg. per 100 ml., respectively. The absolute eosinophil count had risen to 318 per cu. mm., with a drop to 150 after administration of epinephrine.

Sixty-one months after irradiation of the pituitary she weighed 139 pounds (lowest weight was 111 pounds), the blood pressure was 128/78, and the abdominal striae were pale blue with a silvery sheen. She felt symptomatically well and the result was classified as excellent.

Case 4. E.G., white, male

This 17-year-old boy was apparently overweight since early infancy, having started to gain precipitously in his second year following an attack of tonsillitis. At the age of 13, hypertension was discovered. Puberty started about 14. He was admitted to the service of Dr. Calvin Kay at the Hospital of the University of Pennsylvania on May 23, 1950.

On physical examination he was a tall, anxious, perspiring, young man. His height was 5 feet 10 inches, and weight 264 pounds. Obesity was most prominent about the hips and abdomen. There were numerous transverse vivid purple striae across the back and also on the upper thighs and buttocks. The blood pressure varied considerably;

the usual sitting values were 170–190/100–112 mm.Hg. When asleep, his blood pressure was as low as 134/84; when awake, as high as 220/110 mm.Hg.

Roentgen examination of the skull, chest and urinary tract, and body section films of the adrenal region showed nothing abnormal. The benzodioxane test yielded negative results, as did Kolmer and Kline tests and routine urine examination. The hemoglobin reading was 102 per cent, red blood cell count 5 million and white blood cell count 19,700 per cu. mm. The differential count showed 9 per cent lymphocytes, and the absolute eosinophil count was 6 per cu. mm. of blood. The levels of blood urea nitrogen, cholesterol, potassium, and sodium chloride were within normal limits. The basal metabolic rate was −4 per cent. The heart was not enlarged by orthodiogram. The electrocardiogram showed sinus tachycardia, 140 per minute. The glucose tolerance test showed a blood sugar value of 195 mg. per 100 ml. at thirty minutes and 116 mg. at two hours. Urinary excretion of corticoids was 4.2 mg. per twenty-four hours; and of 17-ketosteroids, between 15 and 23.8 mg. per twenty-four hours (within normal limits).

The patient received a total of 4300 tissue roentgens to the region of the pituitary over a period of forty-one days, starting June 2, 1950. One year later there had been a loss of weight to 215 pounds and subsequent slight gain. The purple striae had disappeared, leaving a barely visible silvery sheen. The glucose tolerance test showed a blood sugar value of 147 mg. per 100 ml. at thirty minutes, 166 mg. at one hour, and 108 mg. at two hours. There had been a marked increase in energy and self-confidence. He is now a college student. When last seen, forty-three months after x-ray treatment, improvement had continued but he exhibited a moderate anxiety state. His blood pressure was 148/80 and he weighed 223 pounds. The result has been classified as excellent.

Case 5. M.H., white, female

Five months after the delivery of her third child, Mrs. M.H., a 22-year-old white woman, experienced a weight gain of 40 pounds in one year, as well as increasing fatigability, emotional lability, acne of the face and chest, mild hirsutism (fine hair on her face and increasing hirsutism of her arms and legs), protuberance of the abdomen, and a "puffy" distribution of facial fat. Six months after the onset of her first symptoms, she was found to have hypertension. Cessation of her menses occurred in March 1948, eight months after her initial symptoms.

Dr. John E. Howard of Johns Hopkins Hospital studied Mrs. M.H. in September 1949, at which time she had reddish-purple striae on her buttocks, thighs, abdomen and shoulders, as well as numerous ecchymoses. The systolic blood pressure ranged from 150 to 190 mm.Hg, and the diastolic from 90 to 110 mm. The red blood cell count was 5.9 million per cu.mm. and the hemoglobin level was 16.4 Gm. per 100 ml. A glucose tolerance test revealed fasting, 30-minute, 1-hour and 2-hour blood sugar values of 104, 252, 208 and 168 mg. per 100 ml., respectively. The level of serum sodium was 148 mEq., and of serum potassium 2.8 mEq. per liter. Urinary excretion of neutral 17-ketosteroids was 11.8 mg. per twenty-four hours. The electrocardiogram demonstrated changes compatible with hypokaliemia.

On September 23, 1949, Dr. William Scott of the Johns Hopkins Hospital found both adrenals to be normal at operation. The left adrenal was removed. On microscopic examination of the left adrenal, there were no pathologic changes.

Because Mrs. M.H. moved to Philadelphia, she was referred to the Endocrine Clinic of the Hospital of the University of Pennsylvania for follow-up on November 8, 1949. She received 5250 tissue roentgens to her pituitary over a 52-day period starting November 28, 1949. Her course is shown in Figure 4. Following x-ray treatment she im-

proved markedly, with disappearance of acne, relief of her psychic depression, and return of regular menses (except during two pregnancies terminated by spontaneous miscarriage and therapeutic abortion, respectively. The purple hue of the striae faded to silver. The glucose tolerance curve became flat and the blood pressure remained in a normal range. Six months after termination of x-ray therapy, she had a severe episode of diphtheria, from which she recovered. Fifty-one months after conclusion of her pituitary irradiation, she was menstruating regularly, her blood pressure was 132/92, and she felt and looked well. The result has been classified as excellent.

Comment: There were no signs of beginning improvement in the two months following unilateral adrenalectomy, before the initiation of roentgen therapy. The improvement following radiation did not occur more promptly than would be expected (*see* Table 2). It therefore seems unlikely that the unilateral adrenalectomy was an important factor in her improvement. Sprague (18), in a fairly large experience, has not observed complete remission following unilateral adrenalectomy, nor are we aware of any report of complete remission following this procedure *alone*.

Case 6. A.C., white, female

In November 1936, at the age of 42, sluggishness and puffiness of the face were first noted. Menses, though regular, became scanty. Facial hirsutes and acne appeared. Her basal metabolic rate varied between −15 and −25 per cent, and there was no improvement following the administration of desiccated thyroid. When first examined in May 1937, she had moon-like facies, marked erythema of the face, neck and upper trunk, moderate facial hirsutism and pustular acne over the face, forehead and upper back. The blood pressure was slightly elevated at times. Glucose tolerance was questionably impaired. Roentgenograms showed osteoporosis of the skull, but none was found in the other parts of the skeleton. An intravenous urogram was not remarkable.

Between June 4, 1937, and November 24, 1937, she received two courses of irradiation to the pituitary region with a total tissue dose of 1630 r, and one course of irradiation over the region of the thymus. Menstruation ceased in June 1937. Her weight slowly increased despite efforts at dietary control. By October 1937, moderate hypertension was continuously present. At that time perinephric injection of air showed what was believed to be an enlargement of the right adrenal.

On November 8, 1937, surgical exploration by Dr. E. L. Eliason revealed no gross abnormality of the ovaries or adrenals. In a biopsy specimen from the right adrenal there was "no evidence of hyperplasia or tumor."

During the next six months her general condition fluctuated, with a tendency toward some symptomatic improvement, moderate weight loss and occasional decrease in blood pressure to normal levels. In 1939 hypertension increased, and in 1940 she died after a series of cerebrovascular accidents which followed acute streptococcic cellulitis of the face. Postmortem examination was not made. The results in this case have been classified as poor.

Case 7. S.M., white, male

This 19-year-old man was referred from Delaware Hospital to the Endocrine Clinic of the Hospital of the University of Pennsylvania on April 29, 1952. During the previous three years florid Cushing's syndrome had developed. He exhibited the typical body build and appearance; there were widespread purple striae and also severe miliaria rubra. Examination revealed persistent hypertension (180/130 mm.Hg), severe osteoporosis with several pathologic fractures, and retarded bone age. An oral glucose toler-

ance test (Delaware Hospital, March 5, 1952) showed blood sugar values of 88, 186, 262 and 182 mg. per 100 ml. in the fasting, 30-minute, 1-hour and 2-hour specimens, respectively. No blood eosinophils were found by direct count. Urinary corticoid excretion varied markedly, with values ranging from high-normal to three times that amount. The urinary 17-ketosteroid excretion was within normal limits, as was the excretion of "dehydroisoandrosterone." Repeated roentgen studies showed no evidence of adrenal, pituitary or thymic tumor.

The patient received 1570 tissue roentgens to the pituitary region over a period of twenty-two days beginning June 9, 1952, at the Delaware Hospital. His course is shown in Figure 5. Following this there was disappearance of back pain, weight loss, less fatigue, a slight decrease in blood pressure and distinct improvement in the glucose tolerance curve. On October 1, 1952, the fasting, 30-minute, 1-hour and 2-hour blood sugar values were 86, 150, 139 and 137 mg. per 100 ml., respectively.

An additional course of 1470 tissue roentgens was administered to the pituitary region (at the Delaware hospital) over a period of twenty-three days beginning December 8, 1952. Three months later the glucose tolerance test showed blood sugar values of 81, 142, 123 and 103 mg. per 100 ml. at the usual time intervals. On July 21, 1953, seven months after the second course of irradiation, the blood pressure was 140/110 mm.Hg, the purple striae had decreased considerably in prominence, and the weight was 125 pounds. However, the blood eosinophil count remained low (13 per cu. mm). Although the urinary excretion of corticoids was normal or slightly elevated, the excretion of alpha-ketolic steroids (paper chromatographic methods) was abnormally high. There was no x-ray evidence of improvement in the osteoporosis. He was voluntarily committed to the Delaware State Hospital on May 26, 1953, because of a severe depression, and was discharged from the psychiatric hospital in late September, 1953.

On his last visit on February 16, 1954, he still had some of the stigmata of Cushing's syndrome, but these were far less marked than prior to pituitary irradiation, and his psychiatric status was considerably improved. However, his blood pressure was 150/110. The result has been considered only fair, primarily because of the hypertension, the psychic depression, and continuing laboratory evidence of adrenocortical hyperactivity.

Adrenalectomy has been considered, but thought inadvisable. It is believed that the psychologic and intellectual deficits existing prior to the onset of Cushing's syndrome would make the adrenalectomized state particularly hazardous for this patient.

Case 8. B.B., white, female

This 38-year-old housewife was first seen in August 1948, having been referred by Dr. Harrison Flippin with the diagnosis of Cushing's syndrome. She had had symptoms of moderate severity for at least four years. Her blood pressure was 170/100 mm.Hg. She had a "full," round, red face, a relaxed abdomen with purple striae, a delayed glucose tolerance curve (blood sugar level, 185 mg. at one hour and 137 mg. per 100 ml. at two hours), some hirsutism, and a urinary 17-ketosteroid excretion which was high-normal or slightly above. She had also experienced severe depression. Previously, she had been treated with estrogens. Since stopping these, there had been no menstrual periods for five months.

This patient received three courses of x-ray therapy to the pituitary (Table 1). Following the first course she improved subjectively and there was return of normal menstruation, which continued until her death. Subsequent courses of irradiation did not alter the picture further, even though 3900 tissue roentgens were given over a period of thirty-three days in October and November, 1950, in an attempt to decrease the

hypertension. The hypertension persisted, however, along with the impairment of glucose tolerance and the high-normal or slightly elevated 17-ketosteroid excretion. Facial hair growth decreased somewhat and the purple striae became considerably less marked, but weight was not influenced. A state of apathy and vagueness of thought developed. A cerebrovascular accident occurred in December, 1952, and the patient died after another episode on July 6, 1953—four years and eleven months after her first course of x-ray therapy. The results have been classified as fair.

Postmortem examination in Chestnut Hill Hospital (Dr. Brandt Rose) showed marked atheromatous changes in the circle of Willis, and the lumina of both middle cerebral arteries appeared to have been practically obliterated. Encephalomalacia was evident in the region of the internal capsule and basal ganglia on both sides. The anterior pituitary was "normal." The two adrenal glands weighed 12 Gm. and exhibited slight nodular hyperplasia.

Case 9. E.R., white, male

This 21-year-old man was first treated by pituitary irradiation in July 1932, after a five-year period during which the symptoms developed. At this time, he showed well marked development of the syndrome described shortly before by Harvey Cushing. In addition to the stigmata, he also had marked delay in the glucose tolerance curve, a blood pressure of 150/118 and severe osteoporosis.

Three courses of x-ray therapy to the region of the pituitary seemed to have little effect, but improvement followed a fourth course of 965 tissue roentgens given over a seven-day period in June 1933. In addition, he had also received some irradiation to the "parathyroid region."

Six months later the patient noticed an increase in strength and had lost 8 pounds in weight. By May 1936, his physical appearance had changed considerably; he felt much better and there was x-ray "evidence of re-calcification" in the bones of the skull and thoracic vertebrae. However, he still had hypertension and died (in another city) of cerebral hemorrhage on November 10, 1939, seven years and four months after the initial x-ray therapy.

Autopsy, performed in another hospital, showed no evidence of an adrenocortical tumor. The pituitary was not examined by histologic technics. The results in this case has been classified as fair.

Case 10. F.G., white, female

This 39-year-old housewife was referred to the Hospital of the University of Pennsylvania on June 24, 1937, by Dr. Thomas Fitz-Hugh, Jr., with the diagnosis of Cushing's syndrome. She had had amenorrhea for one year, hypertension for at least one and a half years, (170/100 mm.Hg on admission), moderate facial hirsutism (increasing for the past year), "buffalo"-type fat distribution with a moon face and ruddy cheeks, weakness, recurrent headaches, irritability and depression. A glucose tolerance test showed blood sugar values of 100, 200, 220 and 224 mg. per 100 ml. in the fasting, 30-minute, 1-hour and 2-hour specimens, respectively. There were no purple striae, and no signs of osteoporosis. Many studies were made, including perirenal air-injection studies. No evidence of pituitary, adrenal or thymic tumor could be found.

Starting July 6, 1937, approximately 600 tissue roentgens were administered to the pituitary region over a period of eleven days. She menstruated for two days in August and again one month later; thereafter she had scanty vaginal bleeding once or twice a year until last seen in December 1941. The hypertension and delay in disposition of

glucose remained about the same. Psychic and other symptoms were not improved. The patient refused further x-ray treatment. The response has been rated as poor.

Case 11. R.S., white, female

This 45-year-old housewife was first admitted to the Hospital of the University of Pennsylvania on April 19, 1951, having been referred for special study from the Wilkes-Barre Hospital. During the previous two years, she had experienced the development of increasing hirsutism (sufficient to require daily shaving), chronic leg ulcers, easy bruising, a large relaxed abdomen, and a red rounded face. Hypertension (200/120 mm.Hg) and diabetes mellitus (requiring 30 units of protamine insulin) had been discovered two months prior to admission. There were no purple striae and menses had continued until two months before admission.

Examination and study confirmed the foregoing. There was no evidence of either pituitary or adrenocortical tumor. The 17-ketosteroid and corticoid excretions were slightly above normal on several occasions, and the absolute eosinophil count was 38 per cu. mm. of blood. Numerous other laboratory tests yielded results which were normal or compatible with Cushing's syndrome. Roentgenograms showed severe generalized osteoporosis.

Irradiation to the pituitary region (total dose, 4550 tissue roentgens) was given at the Wilkes-Barre General Hospital over a period of eighty-five days beginning May 10, 1951. She was re-admitted to our hospital on October 9, 1951, with evidence of renal failure and pyelonephritis. There was not the least improvement in the signs of Cushing's syndrome. She became mentally depressed and died suddenly with pulmonary edema on November 6, 1951.

Postmortem examination showed severe necrotizing papillitis and cellular infiltration of the kidneys, left ventricular hypertrophy and widespread arteriolarsclerosis. The adrenals were enlarged (24 Gm.) and exhibited cortical hyperplasia and lipoid depletion. In the anterior pituitary there was an increase in basophilic cells but no definite adenoma. A circumscribed nodule in the right lung field, which had been observed not to change in size on repeated x-ray examination, proved to be a bronchial adenoma. There were also numerous small nodules due to infection with Cryptococcus neoformans. Section of the brain revealed a small recent hemorrhage in the left caudate nucleus. The results in this case have been classified as poor.

Case 12. G.S., white, female

This 30-year-old woman had classic Cushing's syndrome. Her case is reported in detail elsewhere (20), along with studies on urinary and fecal calcium, phosphorus and nitrogen excretion and the effect of testosterone and other agents upon these measurements and her clinical condition.

She had four courses of pituitary irradiation (Table 1), none of which seemed to have a beneficial effect. Examination and biopsies on May 28, 1941, showed both adrenals to be "normal." She survived an attempt at suicide but died on July 19, 1949, ten and a half years after initial irradiation of her pituitary.

Postmortem examination in another hospital showed interstitial hemorrhage in the caudate and lenticular nuclei, benign nephrosclerosis, essentially normal-appearing adrenals, medial sclerosis of the arteries, and a basophilic adenoma of the pituitary. Occasional cells of the anterior pituitary showed hyaline changes. The thyroid gland was "not found." The results have been classified as poor.

THE THYROID HORMONE-PLASMA PROTEIN COMPLEX IN MAN. II. A NEW *IN VITRO* METHOD FOR STUDY OF "UPTAKE" OF LABELLED HORMONAL COMPONENTS BY HUMAN ERYTHROCYTES*†

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SIGNIFICANT advances in elucidation of various aspects of normal and abnormal thyroid function have resulted from the use of I^{131}, chromatography, and chemical quantitation of serum protein-bound iodine levels. The major emphasis in earlier studies was on the thyroid gland itself—its uptake and organification of iodine, and the release into the circulation of a thyroxine-like moiety. In recent studies the focus has shifted somewhat from the gland to such problems as 1) the qualitative nature of the hormone(s)-plasma protein complex, 2) the fate of this complex following its elaboration, and 3) the mechanism of its action on the peripheral tissues. Investigations of these questions have indicated the need for consideration of multiple parameters of thyroid function as an important approach to some of the remaining unsolved problems. The purpose of this report is 1) to present a new method for study of the *in vitro* incorporation of thyroid hormonal components by the human erythrocyte, 2) to summarize results of its use in a variety of clinical conditions, and 3) to evaluate its possible contribution, as another parameter, to our knowledge of thyroid function.

MATERIALS AND METHODS

Donor subjects consisted of healthy technicians and nurses, and patients from the Medical, Surgical and Obstetrical Services, the Endocrine and Prenatal Clinics, and the

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Radioisotope Clinic of the Beth Israel Hospital. Thyroid status was determined, independent of the *in vitro* studies, by clinical evaluation and one or more standard laboratory procedures, *viz*, basal metabolic rate, serum protein-bound iodine level (PBI), 24-hour radioactive iodine (I^{131}) uptake by the thyroid, plasma I^{131} conversion ratio, and blood cholesterol concentration.

For the *in vitro* studies, blood was drawn (with the donor in either a fasting or a nonfasting state) into potassium oxalate[1] (0.1 ml. of a 40 per cent solution per 10 ml. of blood). I^{131} labelled *l*-triiodothyronine[2] (I^{131}-TRI)[1] was made up in isotonic saline to contain $10–120 \times 10–4$ micrograms (μg.) per 0.1 ml.; 0.1 ml. was added, in duplicate, to 3-ml. aliquots of whole blood in a 10-ml. Erlenmeyer flask. Carrier-free I^{131} and I^{131} labelled *l*-thyroxine[2] were similarly employed. The flasks were then shaken at 37° C. for varying incubation periods. At the end of this period, two 1.0-ml. aliquots of whole blood were removed from each flask. With a well-type scintillation counter, the radioactive content of each was determined by counting long enough to obtain ± 2 per cent accuracy. The aliquot was centrifuged, supernatant plasma removed, and the erythrocytes washed five times with ten-fold volumes of isotonic saline. The radioactivity remaining in the erythrocyte fraction was then determined as just described. Alternatively, the radioactivity remaining in the supernatant plasma was measured. The percentile erythrocyte (r.b.c.) "uptake" was then calculated as

$$\frac{\text{net counts in r.b.c.} \times 100}{\text{net counts/ml. of whole blood}}$$

and corrected to an hematocrit reading of 100 for comparative purposes. Uptakes were expressed as an average of the 4 determinations—2 aliquots from the contents of each of 2 duplicate flasks. In a series of "criss-cross" experiments, plasma from 1 donor was combined with the erythrocytes (washed or unwashed) from another compatible donor with the same or different thyroid status and the erythrocyte uptake in this reconstituted blood was then similarly determined.

RESULTS

Following the addition of I^{131} iodide, there was virtually instantaneous equilibration between plasma and red cell. After five washes, however, only 1–2 per cent/100 hematocrit of the total radioactivity was found in the erythrocyte fraction. With I^{131}-THY, there was a slow, progressive disappearance from the plasma. After five washes, however, the two-hour uptake was 1–2 per cent. With I^{131}-TRI there was a greater progressive disappearance from the plasma with strikingly greater erythrocyte uptake at each time interval from ten minutes to four or five hours. For the purposes of this report we present an analysis of the two-hour uptake of I^{131}-TRI. In the vast majority of instances, replicate determinations were within 1–2 per cent of the mean values for each patient when corrected to 100 hematocrit; the standard error of the mean was ± 6 per cent.

[1] In contrast to the differences in *in vitro* "uptakes" by rat diaphragm noted with various anticoagulants (*see* Discussion), there was no apparent difference in erythrocyte "uptakes" between citrated, heparinized or oxalated blood.

[2] Obtained from Abbott Laboratories, North Chicago, Illinois.

I. *Effect of thyroidal status*

The results are summarized in Figure 1.

A. *Euthyroid, with no thyroid gland abnormality.* In 209 subjects, 231 determinations were made. The two-hour erythrocyte uptakes ranged from 10.3 to 17.0 per cent/100 hematocrit and averaged 13.9 per cent.

B. *Euthyroid, with thyroid gland abnormality.* In 29 patients (not included in Fig. 1) with diagnoses of malignancy of thyroid, nontoxic nodular

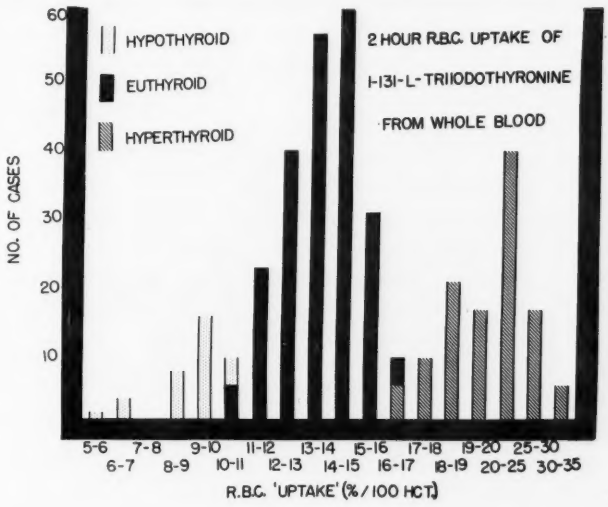

FIGURE 1

goiter or colloid goiter, the uptakes ranged from 10.0 to 16.5 per cent, averaging 13.3 per cent.

C. *Hyperthyroidism.* In 67 patients 110 determinations were made. The uptakes ranged from 16.4 to 34.2 per cent, averaging 21.9 per cent.

D. *Hypothyroidism.* In 29 patients 35 determinations were made. Of the 29 patients, 7 had spontaneous myxedema, 3 were cretins, 3 had hypopituitarism, in 4 the hypothyroidism followed surgery for hyperthyroidism, and in 12 it followed I^{131} therapy for hyperthyroidism. The erythrocyte uptakes ranged from 5.0 to 11.0 per cent, averaging 9.3 per cent. Of the 10 patients with spontaneous and hypopituitary myxedema, only 1 had an uptake greater than 10.0 per cent.

There was some overlapping of the lowest and highest values obtained in euthyroid subjects with the extreme values in hypothyroidism and hyperthyroidism, respectively. However, only 2.6 per cent of all euthyroid uptakes were between 10 and 11 per cent and only 3.8 per cent were be-

tween 16 and 17 per cent. Thus 93.6 per cent of all euthyroid uptakes were distinctive—between 11 and 16 per cent. Further, 95.5 per cent of all hyperthyroid uptakes were greater than 17 per cent, whereas 96.2 per cent of all euthyroid uptakes were below 16.0 per cent.

Although the serum protein-bound iodine levels were similarly distinctive between these three major states of thyroid function, there was no apparent correlation in any single class between the erythrocyte uptake

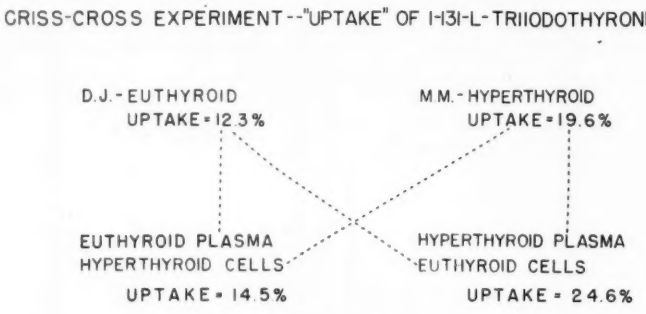

FIGURE 2

and the serum PBI level of the same individual. Similarly, no correlation was apparent between erythrocyte uptakes and basal metabolic rates or 24-hour thyroidal I^{131} uptakes.

Fifty-nine "criss-cross" experiments were performed. A typical experiment is illustrated in Figure 2. In 56 studies, the erythrocyte uptake was determined by the thyroidal status of the *plasma* donor, *i.e.*, the uptake of hyperthyroid erythrocytes in euthyroid plasma was in the euthyroid range, and vice-versa. There were 3 interesting exceptions. In 1, the plasma of a euthyroid sister of a patient with thyrotoxicosis failed to lower the erythrocyte uptake of other hyperthyroid patients; in 2 other euthyroid subjects with a strong family history of thyroid disease, the erythrocyte uptake, although normal in the patient's own plasma, was in the hyperthyroid range when crossed with other euthyroid plasmas.

II. *Effect of induction of euthyroid or hypothyroid state in hyperthyroid subjects*

Repeated studies were carried out in 27 patients when they were euthyroid or hypothyroid following surgical or I^{131} therapy for thyrotoxicosis. In 23, the erythrocyte uptake was consistent with the attained metabolic state as judged clinically and by laboratory tests. In 4, the erythrocyte uptake persisted in the hyperthyroid range for as long as one year after the attainment of the euthyroid (3) or hypothyroid (1) state. As indicated

previously, the uptakes in the hypothyroid patients of this group were higher than those observed in spontaneous myxedema.

III. *Effect of sex, age and season*

Although the uptakes in males were, on the average, higher than in females, (14.3 *vs.* 13.5 per cent in euthyroid subjects, and 22.2 *vs.* 21.6 per cent in hyperthyroid subjects), this difference was not statistically significant in these series by this method. Similarly, although the uptakes were suggestively lower in the older patients (from 50 to 70 years of age), the differences were not statistically significant. There was no apparent difference in uptakes during the various months of the year.

IV. *Effect of pregnancy*

The erythrocyte uptake in 97 observations in 36 pregnant women was strikingly and consistently decreased. The time of onset of this decrease has not been precisely defined because of an insufficient number of studies prior to, and early in pregnancy. However, in 1 patient there was a decrease from 15.3 per cent before pregnancy to 8.5 per cent at approximately the third week of gestation; and in 1 patient there was a decrease from 12.2 per cent to 9.1 per cent between the fourth and seventh weeks. Several women had decreased values between the sixth and seventh weeks, and from this time on, all values ranged between 4.7 and 11.8 per cent, with an average of 7.8 per cent. In each patient, the values remained essentially stable throughout pregnancy and there was no significant difference in the average values for each trimester. Following delivery, values returned to normal in 1 patient on the seventh day, and in several others, between the seventh and fourteenth days. In 2 patients, there was an apparent increase to hyperthyroid values on the fifteenth and seventeenth days.

In 14 of 15 "criss-cross" experiments, pregnancy plasma added to euthyroid erythrocytes lowered the r.b.c. uptake to the pregnancy range, whereas pregnancy cells in euthyroid plasma took up normal or increased amounts of I^{131}-TRI.

In preliminary investigations into a possible hormonal cause for the decreased uptake observed upon the addition of pregnancy plasma, the administration to euthyroid subjects of chorionic gonadotropin or cortisone failed to lower the uptake. Two patients receiving estrogens had decreased erythrocyte uptakes, and a third who was receiving estrogens had a normal uptake which decreased promptly on addition of cortisone therapy.

V. *Effect of exogenous iodine*

Studies were carried out in 23 subjects, from four hours to two months following the administration of iodine-containing compounds (Priodax,

Cholegrafin, Urokon). In 19 subjects the erythrocyte uptakes remained in the euthyroid range despite suppression of thyroidal I^{131} uptake and elevation of the serum PBI level, often to more than 50 μg. per 100 ml. In 4 subjects slight increases were obtained: 1) from 14.9 to 17.3 per cent four hours after intravenous Cholegrafin; 2) from 16.7 to 17.3 per cent twenty-four hours after Priodax; 3) from 17.0 to 18.8 per cent twenty-four hours after Priodax; and 4) from 14.2 to 18.0 per cent twenty-four hours after Priodax and seventy-two hours after intravenous Urokon.

VI. *Effect of dicoumarol*

Following the observation of an increased erythrocyte uptake in a euthyroid patient receiving dicoumarol for treatment of a myocardial infarction, studies were carried out in 12 euthyroid patients before and during therapy with dicoumarol. In each instance there was an increase in erythrocyte uptake, occurring as early as twenty-four to forty-eight hours after institution of therapy with 200–300 mg. of dicoumarol. Values rose from control levels of 11.0–15.4 per cent to 17.8–25.7 per cent. The uptake, once increased, remained essentially stable for as long as twelve days of therapy. No correlation was observed between the erythrocyte uptake and the prothrombin concentration, which progressively decreased during the first week of dicoumarol therapy.

VII. *Effect of administration of iodide or thyroid to euthyroid subjects*

Studies are under way concerning the effects of agents which may invalidate the use of PBI or I^{131} methods. In 10 of 12 euthyroid subjects receiving varying doses of desiccated thyroid, *l*-thyroxine, or *l*-triiodothyronine, erythrocyte uptakes remained in the euthyroid range. In the other 2 patients, after prolonged administration of 3 grains of desiccated thyroid and 0.6 mg. of *l*-thyroxine daily, increased erythrocyte uptakes of 17.3 and 18.7 per cent, respectively, were obtained.

In 1 subject the daily administration of 30 drops of compound solution of iodine (Lugol's) had no effect for ten days; then there was a distinct decrease to low borderline euthyroid values (a fall from 15.9 to 10.6 per cent).

VIII. *Effect of administration of propylthiouracil to hyperthyroid subjects*

To date, 6 patients have been studied. Two of these received 300 mg. of propylthiouracil daily. In one there was a decrease in erythrocyte uptake from 22.0 to 17.4 per cent in twenty-four hours, and then a further decrease to 15.6 per cent in forty-eight hours. In the other there was a decrease from 24.5 to 17.5 per cent in twenty-four hours, and then a further decrease to 14.0 per cent in seventy-two hours.

In 3 of the 6 patients, 300 mg. of propylthiouracil daily caused decreases in seventy-two hours from 18.2 to 14.1 per cent, 22.5 to 13.3 per cent, and 18.0 to 16.0 per cent, respectively.

In the sixth patient (a severely thyrotoxic 24-year-old man), 300 mg. of propylthiouracil daily for two weeks and then 600 mg. daily for another week failed to affect appreciably the 31.8 per cent uptake; this man failed to respond clinically to these dosages and to a subsequent 1,000-mg. daily dosage of this agent.

IX. *Effect of miscellaneous, non-thyroid conditions*

Preliminary observations in small groups of patients with nephrosis ("lipoid" and diabetic), severe hepatitis, marked cirrhosis of the liver, and certain types of extensive metastatic malignancy indicate that the erythrocyte uptake is increased in these patients, who appear euthyroid by other parameters of function.

DISCUSSION

The functional status of the thyroid gland can be accurately assessed for clinical purposes in most patients by the intelligent appraisal of the history and physical examination. Helpful confirmation may result from the judicious use and evaluation of one or more of the currently available laboratory diagnostic methods (BMR, serum PBI level, thyroidal I^{131} uptake, and plasma I^{131} conversion ratio). Final definition may be obtained from the clinical course and the response to a therapeutic trial of desiccated thyroid, iodine, or antithyroid goitrogens.

The clinical usefulness of any single diagnostic method must be evaluated, therefore, in addition to fundamental considerations of accuracy, simplicity and practicability, in terms of 1) its clarification of the status of thyroid function in the minority of patients who present borderline, unusual or bizarre features, and 2) its independence of non-thyroid factors.

The diagnostic accuracy of the *in vitro* incorporation by red blood cells of I^{131} labelled *l*-triiodothyronine added to whole blood compares favorably with that of currently used methods as summarized, for example, by Bauer (1). Thus 96.2 per cent of values in euthyroid subjects were below 16.0 per cent, whereas 95.5 per cent of values in hyperthyroid patients were greater than 17 per cent. At the other end of the spectrum, only 2.6 per cent of euthyroid subjects had uptakes between 10 and 11 per cent, whereas 74.2 per cent of hypothyroid patients had values below 10 per cent. Of 10 patients with spontaneous or hypopituitary hypothyroidism, however, only 1 had an erythrocyte uptake greater than 10 per cent.

The *in vitro* erythrocyte method is simple and rapid. It avoids the ad-

ministration of radioactive material to the patient. It appears to permit assessment of thyroid function under conditions of exogenous iodine administration which prevent the use of serum PBI determinations and thyroidal I^{131} uptake studies. In several borderline cases and in patients who had been treated for hyperthyroidism, results of this test were better correlated with the ultimate definition of thyroid status than were the results of other diagnostic methods. Erythrocyte I^{131} uptake appears to

TABLE 1. EFFECT OF VARIOUS FACTORS ON "UPTAKE" OF I^{131}–TRI BY ERYTHROCYTES

I.	*Decrease*
	A. Pregnancy
	B. Estrogen
	C. Estrogen and cortisone
	D. Propylthiouracil in hyperthyroidism
II.	*No (or slight) effect*
	A. Exogenous iodine
	B. Anxiety
	C. Congestive failure
	D. Hypertension
	E. Mercury administration
	F. Thyroid enlargement—colloid goiter, nontoxic nodular goiter, carcinoma of thyroid
III.	*Increase*
	A. Dicoumarol
	B. Nephrosis
	C. Hepatitis
	D. Cirrhosis of liver
	E. Metastatic malignancy

be of diagnostic value in the face of certain non-thyroid factors which complicate the interpretation of various other methods, *e.g.*, anxiety, hypertension, congestive failure, polycythemia, or the administration of mercury (Table 1). Although a wide experience has been obtained with euthyroid subjects, the number of thyrotoxic and hypothyroid patients has been less extensive. Therefore, the ultimate diagnostic value of this method must await confirmation and extension of these studies.

According to present concepts, circulating thyroxine is preferentially bound to a specific plasma protein(s), characterized by its electrophoretic mobility at pH 8.6, 6.0, and 7.6 between alpha-1 and alpha-2 globulins (2–9). Varying degrees of migration with the albumin fraction have also been reported (8, 9). Triiodothyronine similarly appears to be associated with an inter-alpha globulin fraction, although varying amounts are as-

sociated with several other plasma proteins (10). This binding affinity for triiodothyronine appears to be less strong, for it is readily displaced by thyroxine whereas the converse is not readily effected. The difference in binding affinity has been suggested as a partial explanation of the differences between these two hormonal components in *in vivo* and *in vitro* studies (13–16).

The mechanism and site of incorporation of I^{131}-TRI by erythrocytes in this study remain unknown. The incorporated I^{131}-TRI did not migrate electrophoretically with hemoglobin. Such *in vitro* results may be interpreted in terms of a resultant of relative binding affinities for TRI by the specific binding plasma protein(s), other less specific plasma proteins, and as yet unidentified proteins on the surface or within the tissue (in this instance the red blood cell) (16, 17). Within this concept, the altered erythrocyte uptakes in hyperthyroidism, hypothyroidism, pregnancy, or dicoumarolized plasma may represent quantitative differences in the availability of specific binding sites, depending upon their pre-existing occupation by greater or lesser amounts of thyroxine or triiodothyronine (8, 17). That this concept may not be the entire explanation is suggested by the following considerations:

1. Robbins and Rall (7) were not able to demonstrate a quantitative difference in the thyroxine-binding capacity of serum from 1 hyperthyroid patient compared with sera from 6 euthyroid subjects. We are not aware of similar studies using triiodothyronine.

2. In the present study there was no correlation between serum PBI levels as an index of protein-hormone quantities and erythrocyte uptakes in the given individual in the hyperthyroid, euthyroid, and hypothyroid groups.

3. In previous studies from this laboratory (14) the *in vitro* uptake of I^{131}-TRI by the rat diaphragm was consistently greater from plasma of patients with diffuse toxic goiter than from plasma of (a) patients with nodular toxic goiter with comparably elevated serum PBI levels or (b) euthyroid subjects. The prior enrichment of euthyroid plasmas by the addition of stable THY or TRI to hyperthyroid levels of PBI did not increase subsequent percentage diaphragm uptake of the respective labelled components.

In similar loading experiments with erythrocytes in whole blood, the prior addition of stable THY or TRI did increase subsequent erythrocyte uptake of I^{131}-TRI or I^{131}-THY. This effect, however, increased progressively with increasing time intervals between the prior addition of constant amounts of the stable component and subsequent addition of radioactive label. It may represent, therefore, a true *in vitro* effect of the stable com-

ponent on erythrocyte uptake rather than a "tying-up" of specific binding sites—a process which is not at present considered to be similarly time-dependent.

4. In given subjects the rat diaphragm uptakes were greater from heparinized than from citrated plasma, greater from citrated than from oxalated plasma, and greater from serum than from plasma. These factors are not believed to alter the quantitative thyroxine-protein binding.

5. Recent studies by Recant (18) in nephrosis demonstrated a striking discrepancy between *increased* tissue *in vitro* uptake of labelled hormonal components and *increased* binding capacity of the alpha-globulin fraction for thyroxine (up to 50 μg. per 100 ml.).

6. The 3 exceptions noted in the "criss-cross" experiments in euthyroid subjects and the persistent increased erythrocyte uptake in 3 euthyroid and 1 hypothyroid subject following therapy for thyrotoxicosis are not readily explained solely in terms of available binding sites.

Equally tenable hypotheses within the present framework of knowledge are 1) the possibility of a qualitative difference in the specific binding protein(s) in the different states studied, 2) the effects of other factor(s), such as alterations in protein-protein interrelationships in the plasma in various thyroid states, differentially affecting tissue incorporation of the hormonal components (19), or quantitative differences in the thyroxine-binding globulin. These possibilities are presently under investigation in our laboratories.

SUMMARY

A method has been presented for the study of the *in vitro* incorporation by the human erythrocyte of I^{131}-labelled thyroid hormone components added to whole blood. I^{131}-labelled *l*-triiodothyronine was added *in vitro* to whole blood, incubated, and the radioactivity measured in the subsequently separated, washed red cells as a percentage of whole blood radioactivity corrected to an hematocrit reading of 100.

In diagnostic studies, the two-hour erythrocyte "uptake" of I^{131}-labelled triiodothyronine (a) in 231 determinations in 209 euthyroid subjects ranged from 10.3 to 17.0 per cent, averaging 13.9 per cent; (b) in 110 determinations in 67 hyperthyroid patients ranged from 16.4 to 34.2 per cent, averaging 21.9 per cent; and (c) in 35 determinations in 29 hypothyroid patients, ranged from 5.0 to 11.0 per cent, averaging 9.3 per cent. Of the euthyroid values, 96.2 per cent were below 16 per cent; and of the hyperthyroid values, 95.5 per cent were greater than 17.0 per cent. Of the euthyroid values, 97.4 per cent were greater than 11.0 per cent; and all hypothyroid values were below 11.0 per cent.

The effects of sex, age, season and therapy for hyperthyroidism were

studied. Following administration of non-thyroid organic iodine-containing compounds, there was either no change or a slight increase in uptake. Erythrocyte uptake was strikingly decreased in pregnancy. The oral administration of propylthiouracil caused a prompt decrease (within twenty-four to seventy-two hours) in erythrocyte uptake in 5 of 6 hyperthyroid patients studied. Erythrocyte uptake was increased following administration of dicoumarol, and in nephrosis, hepatitis, hepatic cirrhosis, and extensive metastatic malignancy.

The possible contribution of this method to knowledge of normal and abnormal thyroid function has been discussed.

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SMALL-CELL MALIGNANT LESIONS OF THE THYROID GLAND*†

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IN ALL the varied classifications of malignant thyroid neoplasms, about 20 per cent of the cases are relegated to a subdivision termed "anaplastic" or "undifferentiated." These have perhaps been accorded less detailed study than the other more common groups. This, no doubt, stems partly from the prevailing impression that these lesions are uniformly fatal, and it has resulted in undeserved neglect of certain interesting and important entities. The anaplastic tumors may be broadly divided, on a histologic basis, into large-cell and small-cell types. The reported incidence of the latter varies in different series from 3 to 18 per cent (1, 2). Although previous studies have confirmed the view that no patients with large-cell carcinomas survive any appreciable length of time, several observers have noted the presence of occasional long-term survivors among the small-cell group.

There has long been controversy over the possible existence of primary malignant lymphoma within this group. More recently, speculation has been revived as to a possible association of small-cell malignant lesions with struma lymphomatosa (Hashimoto's disease). Many pathologists, notably Ewing (3), have virtually refused to recognize the existence of primary lymphoma of the thyroid, electing to regard all small-cell lesions as carcinomas. Others have considered malignant lymphoma to be a rarity. In more recent years, in keeping with findings in bone and in the stomach,

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there has been more general acceptance of localized malignant lymphoma as an entity. In 1953 Brewer and Orr (4), in a comprehensive review of the literature, found 34 acceptable "reticulo-sarcomata" of the thyroid and added 13 new cases. Since then at least 15 other cases of primary malignant lymphoma have been reported in the literature. The widely divergent views as to the proper classification of these lesions effectively prevent any accurate statistical assessment of various series by observers unfamiliar with the material under review.

Whatever the classification adopted, however, physicians generally have regarded the prognosis of small-cell neoplasms with unrelieved pessimism. Of the 8 patients who had lymphosarcoma of the thyroid reported by Dinsmore and associates (5), all were dead within seven months. Lindsay and Dailey (6) observed 1 of 8 patients alive longer than five years. On the other hand, in a group of 35 patients with small-cell malignant lesions (33 of whom were felt to have carcinoma) McDermott and associates (2) had 5 five-year survivors, all regarded as having carcinomas. Brewer and Orr (4) reported 8 five-year survivors in a group of 47 patients having reticulosarcomas whose cases they reviewed. The discrepancy among the published results of different observers reflects the wide range of differing opinion on the prognosis of small-cell malignant lesions. Under these circumstances, it is possible that unjustified pessimism may lead to unwitting neglect of potentially curable lesions.

In an effort to elucidate any special histologic or prognostic features, all small-cell neoplasms of the thyroid gland seen at the Mayo Clinic in a 26-year period (1929–1954 inclusive) were reviewed.

MATERIAL AND METHODS

To select these cases, the microscopic slides of all malignant neoplasms of the thyroid gland seen at the clinic between 1929 and 1954 inclusive were studied. From these, all tumors which the average observer would reasonably regard as of "small-cell type" were selected, provided the areas of malignancy were not papillary, follicular or spindling in pattern. Any case in which doubt existed as to the thyroidal origin of the tumor was rejected. No case was included in which the thyroid was involved from without by tumor or was associated with recognizable generalized malignant lymphoma. Thirty patients who satisfied these criteria were found and carefully studied. In addition, the slides of all cases diagnosed as struma lymphomatosa or "lymphocytic thyroiditis" between 1940 and 1954 inclusive were reviewed, in an effort to obtain a clearer picture of the morphologic variation within this group and to determine whether any cases of struma lymphomatosa might be confused histologically with small-cell carcinoma or malignant lymphoma of the thyroid.

PATHOLOGIC DIAGNOSIS OF MALIGNANT LYMPHOMA AND SMALL-CELL CARCINOMA

Despite the controversy in the past and occasional denial by some authors of the existence of such a lesion, we believe that primary lymphoma of the thyroid gland is a definite entity, which is usually not difficult to diagnose on histologic grounds alone. The general architecture of the tumor and the cytologic picture resemble very closely the features of malignant lymphoma as seen in other sites, notably lymph nodes. Furthermore, the histologic appearance of the thyroidal tumor diagnosed as primary malignant lymphoma appears identical with that of the thyroidal tumor observed as part of a proved generalized lymphoma.

The malignant lymphomas of the thyroid in this series all fell within the category of lymphosarcoma or reticulum-cell sarcoma (Fig. 1). There were no examples of Hodgkin's disease or giant-follicle lymphoma. In the thyroid gland, as in malignant lymphoma elsewhere, there is a wide range of variation from the mature type of lymphosarcoma characterized by sheets of small, uniform, neoplastic dark cells resembling lymphocytes, to the more malignant-appearing reticulum-cell sarcoma, with its larger cells, greater pleomorphism and more numerous mitotic figures. Only 3 cases in this series were classified as mature or lymphocytic lymphosarcomas (Fig. 1b). In the remainder, various gradations among the immature lymphosarcomas (Fig. 1, c and d) were found, making the finer distinction between these groups difficult in a certain number of cases.

Twelve tumors in this series were diagnosed as small-cell carcinoma (Fig. 2) on the basis of the following histologic features: The histologic pattern lacked the completely uniform "monotonous" low-power appearance of the lymphomas, and the cells were arranged in small or large nests, clumps or alveoli surrounded by fibrous stroma (Fig. 2, b and c). In addition, the individual cells in the carcinoma group appeared more hyperchromatic than in the malignant lymphomas, with usually more variation in size and shape of cells.

The patterns of invasion of adjacent thyroid parenchyma in malignant lymphoma and in carcinoma differed somewhat, although this distinction was not invariable. The malignant lymphomas grew in such a manner as to leave very little thyroid tissue within the main body of the tumor. Frequently, a zone interpreted as lymphocytic thyroiditis surrounded the main tumor mass, and beyond this zone was normal thyroid parenchyma. In contrast, the carcinomas appeared more "aggressive" and tended to engulf and leave behind circumscribed islands of normal-appearing thyroid tissue. There was less associated lymphocytic infiltration surrounding the carcinomatous tissue.

The presence or absence of necrosis could not be used as a distinguishing

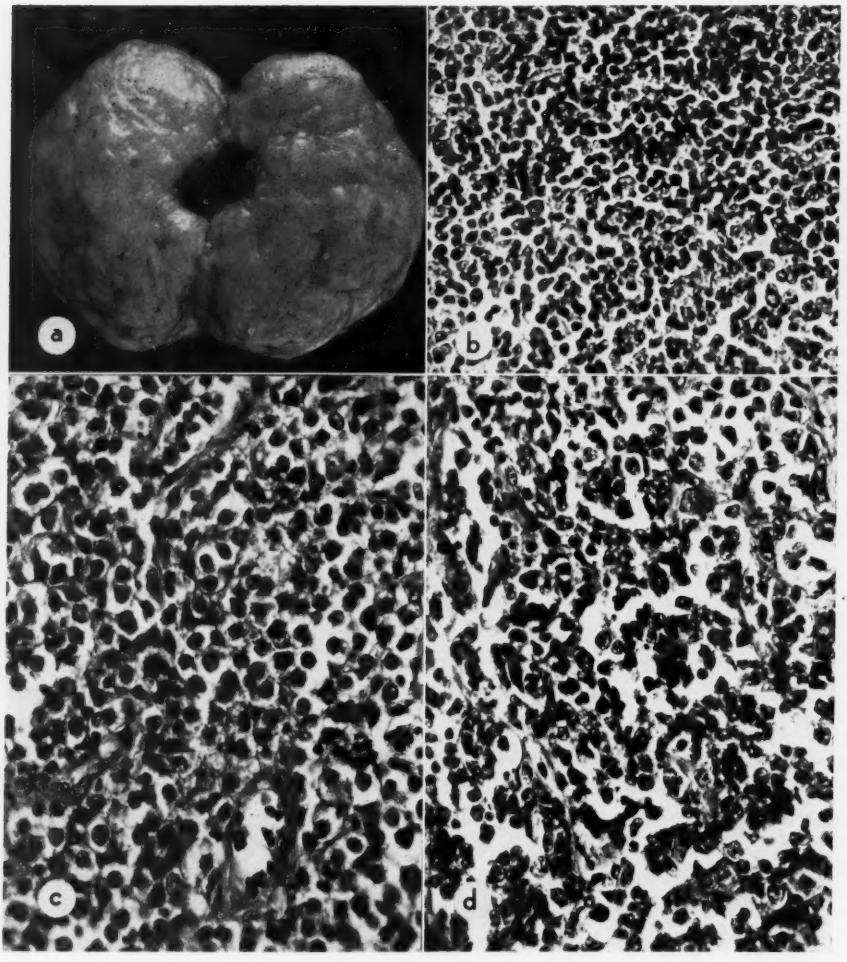

FIG. 1. Primary malignant lymphoma of the thyroid gland. **a.** Operative specimen of immature type of lymphosarcoma, weighing 300 Gm. **b.** Mature type of lymphosarcoma (×350). **c.** Immature type of lymphosarcoma with larger cells and mitotic activity (×375). **d.** Immature lymphosarcoma, showing gradation between **b** and **c** (×375). In all figures the stain is hematoxylin and eosin.

feature. Although it was more commonly seen in carcinomas, some cases of malignant lymphoma also exhibited this feature.

The gross appearances of carcinoma and lymphoma were not sufficiently dissimilar to be diagnostic. Most malignant lymphomas were limited to one lobe, which was replaced to a greater or lesser extent by well-demar-

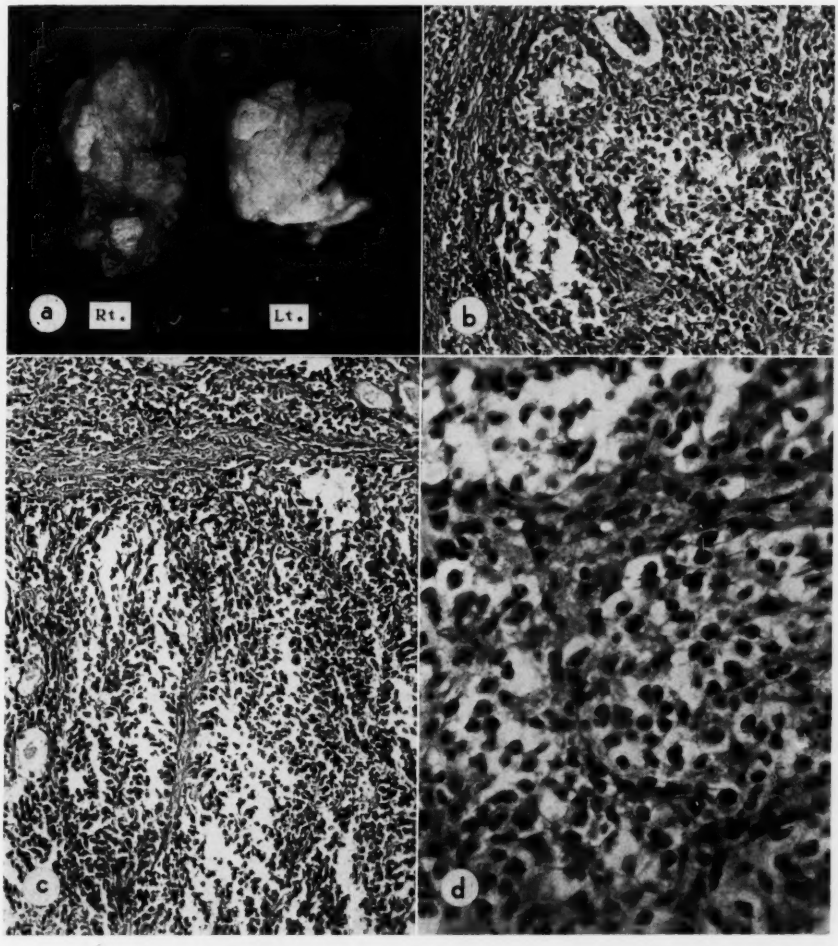

FIG. 2. Small-cell carcinoma of the thyroid. **a.** Gross appearance, showing bilateral diffuse involvement. **b.** Histologic section of small-cell carcinoma. The carcinoma cells are arranged in clumps surrounded by fibrous stroma (×140). **c.** Small-cell carcinoma. Note fibrous trabeculae (×140). **d.** High power (×375).

cated, soft, fleshy, tan-colored tumor tissue (Fig. 1a). Actual infiltration of extrathyroidal tissues occurred in a few instances. In general, the carcinomas appeared more invasive than the lymphomas and were considered "inoperable" in a high percentage of cases. In 7 of the 12 carcinomas, biopsy specimens only were removed.

Reticulin stains, although not diagnostic in themselves, were helpful, in that they accentuated the histologic differences noted in preceding

paragraphs. In general, in lymphosarcoma the reticulin fibers formed a delicate network surrounding individual malignant cells or small groups of malignant cells. In the carcinoma group, on the other hand, the reticulin fibers tended to surround larger masses of cells, within which there was complete absence of the reticulin network (Fig. 3, a and b). These special stains were felt to be of value only when the findings lent support to a diagnosis based on the morphologic features described earlier.

MALIGNANT LYMPHOMAS

Of the 18 cases classified as malignant lymphomas, 3 were of a mature cell type, ordinarily regarded as lymphocytic lymphosarcoma. In the remaining 15 cases, the cell type was less mature, ranging from lymphoblastic lymphosarcoma to reticulum-cell sarcoma. Because of the apparent gradation, we did not feel justified in drawing a distinct line between these immature lymphosarcomas and reticulum-cell sarcomas, but preferred to classify all as immature lymphosarcoma.

Age and sex. The patients ranged in age from 33 to 79 years, the average age being 56 years. The average age of women was 55 years, of men 58

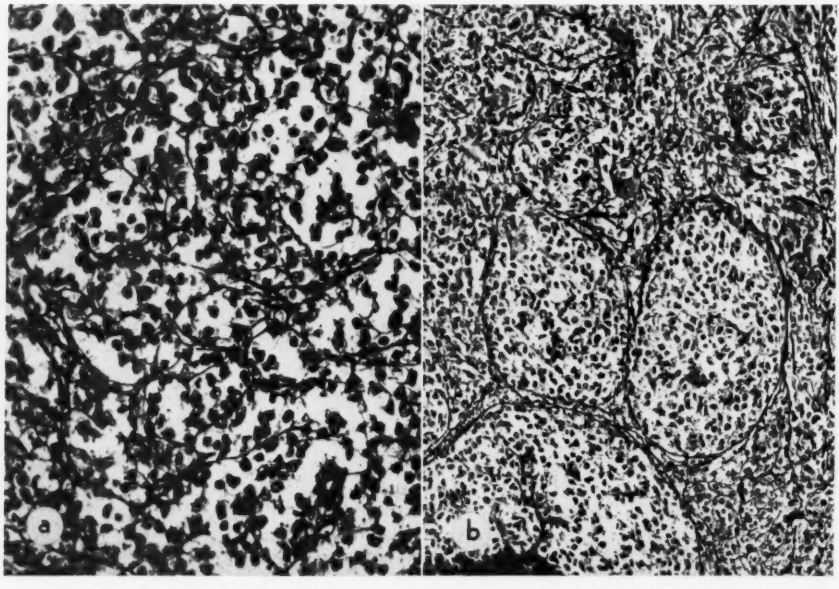

FIG. 3. Reticulin stains. **a.** Lymphosarcoma, immature type, showing delicate reticulin network (×265). **b.** Small-cell carcinoma, showing larger cell masses surrounded by reticulin framework (×135).

years. Only 5 of the group were less than 50 years of age. Thirteen of the patients were women and 5 were men.

Pre-existent goiter. With the exception of 1 patient, in whom the goiter was detected in the course of a routine physical examination, all patients were aware of a swelling in the neck. In two thirds of these the swelling had been noted less than two months before examination, and in most of them any increase in size had been gradual. In the few in whom enlargement had been rapid, the outcome in retrospect was bad.

Clinical picture. In 1 case the goiter was found incidentally in the course of a general examination. In 10 cases the goiter alone precipitated the patients' decision to seek medical advice. The remaining 7 patients presented a variety of signs and symptoms, as shown in Table 1.

TABLE 1. MAIN SYMPTOMS AND SIGNS IN 18 PATIENTS WITH MALIGNANT LYMPHOMA

Sign or symptom	Patients
Presence of a goiter	17
Tenderness	5
Dysphagia (usually slight)	4
Dyspnea (usually slight)	3
Voice changes	3
Fixed vocal cord	2
Pain in thyroid area	1
Significant loss of weight	0

In 13 cases a preoperative diagnosis of a malignant lesion was easily made. This was based solely on physical features of the gland in most cases, but in the more advanced cases it was reinforced by the presence of a fixed cord or suspicious degree of dyspnea and discomfort of the patient.

The physical characteristics of the affected glands were determined by the stage of the disease. Unusual firmness was often noted, but nodularity was rarely present, the gland or lobe tending to preserve its normal contour. In those cases in which the tumor was found to be resectable, its weight varied between 30 and 100 Gm., with 3 exceptions, in which 210 Gm., 215 Gm. and 300 Gm. of tissue were removed. In 4 cases the gland was distinguished by its marked fixation, due to gross infiltration of adjacent tissues.

In 5 of the 18 cases the preoperative diagnosis was less obvious. In these, the probability of Hashimoto's disease was entertained in 2 and that of benign adenomatous goiter in another 2. In 1 case the diagnosis of malignancy was regarded as equivocal.

Operative findings and treatment. These naturally reflect the stage of progression of malignant lymphoma at the time of operation. In the ideal case, in which the tumor was still localized to the gland, it presented most often as a well-circumscribed mass of varying size. On cut section it was characterized by its uniform, fleshy, rubbery, somewhat yellowish surface. In a few there were obvious areas of necrosis. At operation, in 8 cases there was unmistakable evidence of infiltration of the perithyroidal tissues, chiefly the strap muscles, by the lymphomatous process. In 3 of these there was also infiltration of the trachea, the esophagus, or both.

Several surgical procedures were employed in the treatment of these lesions. The 18 operations—all followed by deep roentgen therapy, radium, or both—were as shown in Table 2.

In contrast to small-cell carcinomas of the thyroid (*see* later), in which 7 of 12 cases were considered suitable merely for biopsy or removal of the isthmus for the purpose of inserting a tracheotomy tube, only 3 of 18 patients who had lymphoma had reached this pass. This probably indicates the relative malignancy of these lesions. The remaining 15 lymphomas were amenable to the more definitive procedures stated in Table 2.

TABLE 2. PRIMARY MALIGNANT LYMPHOMA OF THYROID

Patient			Operation on thyroid*	Present state	Post-operative survival (yrs.)
Case No.	Sex	Age (yrs.)			
1	F	33	Bilateral subtotal thyroidectomy	Dead	$\frac{1}{12}$
2	M	51	Biopsy	Dead	$\frac{2}{12}$
3	M	42	Unilateral subtotal lobectomy	Dead	$\frac{3}{12}$
4	F	39	Unilateral subtotal lobectomy	Dead	$\frac{4}{12}$
5	F	75	Bilateral subtotal thyroidectomy	Dead	$\frac{10}{12}$
6	F	63	Biopsy	Dead	1
7	F	58	Bilateral subtotal thyroidectomy	Dead	1
8	M	37	Removal of isthmus	Dead	$1\frac{1}{2}$
9	F	76	Unilateral lobectomy	Dead	$1\frac{11}{12}$
10	M	67	Total thyroidectomy	Dead	$2\frac{1}{12}$
11	M	79	Bilateral subtotal thyroidectomy	Dead	7
12	F	50	Unilateral lobectomy	Alive	$11\frac{1}{2}$
13	F	52	Unilateral lobectomy	Alive	$6\frac{3}{4}$
14	F	62	Unilateral lobectomy	Alive	6
15	F	60	Unilateral lobectomy	Alive	6
16	F	71	Bilateral subtotal thyroidectomy	Alive	$5\frac{1}{2}$
17	F	41	Total thyroidectomy	Alive	4
18	M	53	Subtotal thyroidectomy	Alive	$2\frac{3}{4}$

* Postoperative irradiation in all cases.

Prognosis. Among the 18 patients reviewed, there were 11 deaths—1, 2, 3, 4, 10, 12, 12, 18, 23, 25, and 84 months after the first appearance at the clinic. A patient (a man, aged 79 years when first seen) who survived seven years and who was said (after necropsy) to have died of a coronary occlusion is known to have had a recurrence treated with radium at the clinic two years before he succumbed. Judging from a follow-up letter shortly before his death, it is probable that he had a re-recurrence at the time of death, although there was no mention of this in the coroner's report. It is noteworthy that, at the original operation, total lobectomy was performed on the right side and subtotal lobectomy on the left, since the left lobe was only slightly enlarged and was thought to be free of malignant tissue. When the goiter reappeared five years postoperatively, it did so on the side of the subtotal lobectomy. External irradiation in this particular case produced at least temporary regression of the recurrent mass. In contrast, a 71-year-old woman, in whom a recurrence of goiter developed one year after bilateral subtotal thyroidectomy in which 215 Gm. of tissue had been removed, responded exceedingly well to external irradiation and is well and completely asymptomatic six years after her original operation. All patients with voice changes were dead within eighteen months.

Seven patients are alive without symptoms, $11\frac{1}{2}$, $6\frac{3}{4}$, 6, 6, $5\frac{1}{2}$, 4, and $2\frac{3}{4}$ years postoperatively. Of the 6 patients who have survived four years or more, 4 underwent removal of the isthmus and subtotal lobectomy on the affected side. One underwent total thyroidectomy, and 1 bilateral subtotal thyroidectomy.

Of the 8 patients in whom the tumor had infiltrated adjacent structures, 5 were dead within two to 18 months. Three patients in whom there was marked infiltration of surrounding tissue, however, were alive and well 83, 71, and 32 months after treatment consisting of total unilateral thyroidectomy, with removal of the isthmus in 2, bilateral subtotal thyroidectomy in 1 and subsequent radiotherapy in all 3. In 2 of these 3 cases, muscle had to be removed in continuity with the tumor owing to direct invasion. These findings should stimulate the application of maximal resection even in discouraging circumstances.

SMALL-CELL CARCINOMAS

Twelve cases of primary small-cell carcinoma were found in the group of 30 small-cell lesions of the thyroid.

Age and sex. The patients ranged in age from 27 to 83 years, and only 2 were less than 40 years of age. The average age was 53.5 years. Ten were women, and 2 were men.

Pre-existent goiter. Six patients had noticed a goiter for four years or

longer. (Three claimed to have had goiters for thirty to forty years.) Five patients had felt their goiters for periods varying from a year to two months. Only 1 had no previous knowledge of his lesion.

In many there had been sudden steady enlargement, often for three to four months before they presented themselves. In most, although the speed and degree of enlargement naturally varied a fair amount, it was the presence of the goiter rather than other complaints that precipitated their visit to the clinic.

Clinical picture. Associated symptoms among this group were as shown in Table 3.

Loss of weight and pain in the region of the thyroid were virtually absent, and the dyspnea and dysphagia in this series were seldom marked

TABLE 3. SIGNS AND SYMPTOMS IN 12 PATIENTS WITH SMALL-CELL CARCINOMA

Sign or symptom	Patients
Hoarseness	5
Cord abnormality	
Fixed	1
Weak	2
Dysphagia	5
Stridor and dyspnea	4
Cough	3
Pain	
Bones	2
Chest	1

when these patients were first seen. Tracheotomy was performed twice, once prophylactically and once as an emergency procedure.

Hoarseness, wheezing and cough were observed in 6 patients. In 3 of them, these symptoms persisted in the presence of normal vocal cords. At operation, the trachea was found to be involved, by pressure or infiltration. In 1 patient, a cord was fixed, and in 2 poor movement was reported.

Pain due to metastatic involvement was subsequently present in 3 patients. Two of these had osteolytic lesions, widespread in one, and apparently limited to the sixth cervical vertebra in the other, with associated root pains. The third patient had pulmonary spread.

The diagnosis of a malignant lesion was very obvious on clinical grounds in all 12 cases. Many of the glands were large, nodular and fixed, and all were of a hard consistency on palpation.

Operative procedures. The operative procedures carried out on this group were primarily for palliation. In 7, there was only biopsy of the gland or exposure of the trachea to help obviate obstruction. In 3, unilateral subtotal thyroidectomy and in 2, unilateral total and contralateral subtotal thyroidectomy were performed. The more extensive operative procedure (with subsequent radiotherapy) did not prolong the survival time, for some patients who underwent only biopsy and irradiation were among the longest survivors, whereas those who had extensive treatment often succumbed rapidly.

Survival. All 12 patients are known to be dead, two to nineteen months after they were first seen at the clinic.

The average survival time in this group of patients who had small-cell carcinoma was nine months, but almost half lived a year or more. This is a somewhat longer average survival time than has been noted by other observers.

THE RELATIONSHIP OF STRUMA LYMPHOMATOSA TO SMALL-CELL MALIGNANT LESIONS

In view of recent interest in the possible association of struma lymphomatosa and small-cell malignant lesions, our material was reviewed from this aspect. It is vital in any such discussion to define one's concept of struma lymphomatosa, since there appear to be important variations in the strictness of the criteria demanded. These are reflected in subsequent statistical deduction. The mere presence of large numbers of lymphocytes or focal groups of follicles exhibiting epithelial change does not constitute struma lymphomatosa. The basic changes in the thyroid in this condition consist of 1) diffuse epithelial degeneration, in which the acinar cells frequently show marked eosinophilic features; 2) varying degrees of lymphocytic and plasma-cell infiltration, with widespread germinal-follicle formation in some; and 3) variable amounts of fibrosis. It should be stressed, however, that there is no single characteristic pattern, but rather a wide range of histologic variation, depending on the relative dominance of each of these features. In some cases, the fibrosis is striking; in others, epithelial change is typical and widely distributed. In yet others, the small-cell infiltration may overshadow these completely. Nevertheless, all these features are present to some degree in any one case. Adopting these strict criteria, the parenchyma adjacent to the tumor was examined in the 18 malignant lymphomas in this series. The histologic findings in the part of the thyroid not involved by tumor are outlined in Table 4.

The 2 cases of struma lymphomatosa, in which the patients were women aged 58 and 76 years, were unequivocal examples of this condition and fulfilled all the criteria outlined in the preceding paragraph. Much of the difficulty in assessing the true incidence of the association of struma lymphomatosa and malignant lymphoma lies in the varying interpretation of the group of "lymphocytic thyroiditis" by different observers. Variable numbers of lymphocytes are commonly observed in otherwise normal glands, and this feature becomes more prominent in the older age group.

TABLE 4. HISTOLOGIC FINDINGS IN THE PART OF THE THYROID NOT INVOLVED BY TUMOR IN 18 PATIENTS WITH MALIGNANT LYMPHOMA

Histologic finding	Patients
Inadequate tissue for sampling	4
Normal thyroid	3
"Lymphocytic thyroiditis"	9
Struma lymphomatosa	2
Total	*18*

Unfortunately, a fairly narrow zone of cellular infiltrate is frequently prominent on the periphery of the advancing tumor; and this, in the absence of sufficient available tissue from farther afield, has been regarded by some as representative of the whole gland and thus interpreted as struma lymphomatosa. In a few cases there may be small scattered foci of epithelial change which, when viewed without regard to the normal appearance of intervening thyroid tissue, have also apparently led to the loose diagnosis of struma lymphomatosa. We have preferred to regard the frequently observed but limited changes of this kind as "focal lymphocytic thyroiditis" and do not consider these to be necessarily related to struma lymphomatosa. In those series in which these minimal or incomplete histologic features have been accepted as adequate for the diagnosis of struma lymphomatosa, the reported incidence of struma lymphomatosa in cases of small-cell malignant lesions suggests a definite association of these entities. We believe this to be based on a deceptively high estimation of the true occurrence of struma lymphomatosa.

Recognizing the need to determine the approximate incidence of struma lymphomatosa in our own material, the thyroid in each surgical case diagnosed as chronic lymphocytic thyroiditis in the fifteen years between 1940 and 1954 was examined (Table 5).

The percentage of resected goiters diagnosed as affected by struma lymphomatosa (1.2 per cent) is in close agreement with the experience of

TABLE 5. INCIDENCE OF STRUMA LYMPHOMATOSA

	Approximate number	Per cent
Thyroidectomy (1940–1954)	13,600	100
Chronic lymphocytic thyroiditis (exclusive of exophthalmic goiter)	600	4.4
Struma lymphomatosa	160	1.2

Joll (7), of Statland and associates (8), and of others; but it is appreciably lower than the 7.1 per cent recorded by Chesky and associates (9) and the 5 per cent recorded by Lindsay and associates (10). Struma lymphomatosa formed about a quarter of the cases of chronic thyroiditis in our series, an incidence comparable with the 21 per cent reported by Marshall and Meissner (11) of the Lahey Clinic.

COMMENT

In the past, there have been few careful studies of small-cell malignant lesions of the thyroid, mainly because of their relative rarity and the lethal prognosis attributed to them. The series reported here reinforces some old views on the group but exhibits certain features which do not justify the gloomy impressions that have uniformly prevailed until recent years. It is important that the distinct group of primary malignant lymphomas be recognized, in view of the reasonably favorable prognosis associated with them. This is not peculiar to malignant lymphomas in this organ but brings them into line with similar lesions found in the stomach and in bone, and with malignant lymphoma localized to cervical nodes.

A predominance of women is evident in all series of thyroid malignant lesions. In Brewer and Orr's (4) collected series of cases of reticulosarcoma only 4 of 47 patients were male. Among the malignant lymphomas in the present series, however, this distribution was not as pronounced as in Brewer and Orr's series, since 5 of the 18 patients were male. This does not bear out Anderson's (12) statement that lymphomas, when primary in the thyroid, appear almost exclusively in elderly women. Furthermore, about a quarter of the patients were less than 45 years of age. Age and sex, therefore, do not constitute reliable diagnostic criteria.

The preoperative recognition of the malignant nature of these lesions is not difficult in most cases. It is unfortunate that almost all of the small-cell carcinomas and a few of the lymphomas are so advanced when first seen that unmistakable gross signs of malignancy are already present. Actual identification of the type of malignancy can be made only on microscopic examination. It is important that this examination be made accurately,

because of the influence it may have on the extent of treatment prescribed, in view of the differing prognoses of the two types.

Surgery has a definite place in the treatment of these lesions. It is essential for the confirmation of the diagnosis, and thereafter may be directed toward palliation or toward cure. In the more advanced cases, biopsy and subsequent tracheotomy may be performed prophylactically as an adjunct to intensive irradiation, or, in a few unfortunate patients, as an emergency measure. The small-cell carcinomas do not appear to be susceptible to cure by any method available at present, but in those patients in whom widespread infiltration does not preclude the possibility, resection should perhaps be performed in the hope of prolonging survival.

Malignant lymphomas present a far more hopeful outlook. There is reason to believe, from this series of cases, that local resection followed by irradiation may produce apparent cure, or at least long-term survival, in an appreciable number of cases. The extent of this resection must vary with the degree of thyroid involvement at the time of operation. On the basis of available evidence, it would seem that, at the very least, total lobectomy on the involved side with removal of the isthmus should be performed. Since, on gross inspection, one cannot always exclude the presence of tumor in the other lobe, it is probable that routine subtotal lobectomy should be added, and only enough thyroid tissue preserved to protect the parathyroids on that side.

Irradiation is invaluable in producing palliation in some cases. Subsequent metastatic lesions in the malignant lymphoma group have been observed to regress markedly or even disappear temporarily after irradiation. In at least 2 necropsy cases, there was no evidence of viable tumor after four to eight months of irradiation of unresected areas of malignant lymphoma in the thyroid. Local recurrences may respond dramatically. Since all the long-term survivors in the present group underwent irradiation following their surgical treatment, it is thought advisable to continue this program routinely.

Small-cell carcinomas respond far less satisfactorily. In this series, despite radiotherapy, no patient with this lesion survived longer than nineteen months. It is possible, however, that there may have been prolongation of life, since some of the goiters definitely receded in size after radiotherapy.

SUMMARY AND CONCLUSIONS

1. All primary malignant neoplasms seen at the Mayo Clinic between 1929 and 1954 were reviewed. Among them, 30 small-cell malignant lesions were found. These were divided histologically into two distinctive groups,

which consisted of 18 malignant lymphomas and 12 small-cell carcinomas.

2. The average age of patients with malignant lymphoma was 56 years and of those with small-cell carcinoma 53.5 years. These lesions are uncommon below the age of 40 years.

3. As in all groups of patients with thyroid neoplasms, there was a predominance of women. Thirteen of the 18 patients with lymphoma, and 10 of the 12 with small-cell carcinoma were women.

4. The need to adopt strict criteria in the diagnosis of struma lymphomatosa before speculating on any possible association with small-cell malignant lesions is stressed. Unequivocal struma lymphomatosa was present in 2 of 14 cases of malignant lymphoma in which sufficient tissue for adequate sampling was present, but this series is too small to lend itself to any firm conclusions as to possible relationships.

5. The prognosis in the group of malignant lymphomas was much better than among the tumors designated as small-cell carcinomas. In the former series, 6 of 18 patients were alive and asymptomatic four years or more after they were first treated. In the carcinoma group, no patient survived longer than nineteen months.

6. Apparent cure in cases of lymphoma of the thyroid may occur after unilateral or bilateral thyroidectomy followed by radiotherapy. It would appear that surgical excision should always be followed by irradiation, until more knowledge is available.

7. Surgical treatment of malignant lymphoma of the thyroid should be vigorous and should not be abandoned even in the face of local attachment to muscle or peritracheal and periesophageal tissue.

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THYROID FUNCTION STUDIES IN CHILDREN: NORMAL VALUES FOR THYROIDAL I^{131} UPTAKE AND PBI^{131} LEVELS UP TO THE AGE OF 18*

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RADIOIODINE has been used in normal children to study thyroid function. All but one of the previous studies concerning children have been limited, in that only the concentrating ability of the thyroid for iodine has been tested. This function was measured as the percentage thyroidal uptake of the administered dose at twenty-four hours (1–6) or at forty-eight hours (7). In one study (8), the secretion rate of protein-bound radioiodine (PBI^{131}) of serum was measured at various intervals after I^{131} administration, but there was no information about the age groups tested.

This study was undertaken to establish normal standards for thyroidal uptake and serum PBI^{131} values at various ages and to clarify the discrepancies in normal uptake values reported from various parts of the United States.

The data presented indicate that the thyroid gland in children up to the age of 4 years, and possibly later, is normally in a state of "hyperactivity" when compared to that of the euthyroid adult.

METHODS

Selection of subjects

The children used in this study were from the patient population of Sarah Morris Hospital for Children and the Mandel Clinic of Michael Reese Hospital. Eighty-three children were selected whose ages ranged from $2\frac{1}{2}$ months to 18 years; 61 were considered normal so far as thyroidal status was concerned, and 12 had various thyroid disorders. Ten were tested but were not included in this study for the following reasons:

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3 were receiving thyroid therapy up to four days before the test, 1 was receiving large doses of hydrocortisone, 1 had anorexia nervosa, 3 recently had received iodine-containing compounds (dye for intravenous pyelograph, Lugol's solution, cod liver oil), and 2 had recent concussion injuries and had low uptakes.

A child was considered normal if growth and development were normal, if there was no history of recent interfering medications or thyroid disorder, and if there were no signs of acute or chronic infection at the time of the study. In the normal group, studies of thyroidal I^{131} uptake were omitted in a 3-year-old child, and of serum PBI^{131} in an 18-year-old girl, thus reducing the number of children in this group to 60 when considering either of these parameters separately.

Five children admitted for acute infections were tested three to ten days after symptoms had disappeared. Four children were observed five to seven days following a surgical operation. In the adult, fever and most illnesses do not appear to alter the thyroidal uptake or I^{131} turnover (9), and acute illnesses or surgical procedures do not appear to alter the serum protein-bound iodine level (10).

Most of the normal children were seen for various disorders, as shown in Table 1. Children with thyroid disorders are listed in Table 2. Sixty-four euthyroid adults (normal diet, no thyroid enlargement, no previous thyroid surgery or I^{131} therapy) and 62 hyperthyroid adults previously studied are included for comparison (Table 3).

Procedures

Children up to the age of 10 years received 22–25 microcuries (μc.) of I^{131} as a test dose; those above that age were given 50 μc. The radioiodine was given in capsule form.[1] On the morning of administration, breakfast consisted of milk, buttered toast, and jelly for the younger children; for the adolescents, coffee with cream was substituted for milk when requested. The diet for two days prior to, and during the testing was devoid of all seafood and highly seasoned snacks. This precaution was taken in order to keep the iodide content of the diet low.

(a) *Thyroidal uptake measurements.* The children were in a sitting position in front of a scintillation counter.[2] Readings lasting three minutes each were taken at two distances in order to minimize the errors due to geometry (50 and 70 cm. from the center of the counter to the estimated center of the thyroid gland) (11). Net readings at these distances were in the ratio of 2:1. A third reading was taken at 50 cm. with a lead shield (1 inch thick) in front of the child's neck. This reading represented background radioactivity from the tissues and the room; when it was subtracted from each of the gross thyroid readings, the result was the net thyroid reading at each distance. On the day of counting, a capsule identical in strength to that dispensed to the subject was counted in a phantom neck (made of aluminum) at the same two distances. The phantom had a cylindrical opening admitting a test tube containing the standard capsule. The center of the opening was 3 cm. from the front of the phantom. In all cases studied the geometrical ratios (2:1) were within the statistical accuracies of the readings. The ratio between the net readings of the subject and those for the standard multiplied by 100 indicated the percentage uptake corrected to zero time.

The scintillation counter contained a $1\frac{1}{2}$-inch $\times$ 1-inch sodium iodide (Th) scintilla-

[1] Obtained from Abbott Laboratories, through license from the U. S. Atomic Energy Commission.

[2] The scintillation probe and well-counter were obtained from Nancy Wood Counter Laboratories.

TABLE 1. I^{131} DATA ON NORMAL CHILDREN

Patient					Thyroidal I^{131} uptake (% dose)				Serum PBI^{131} (% dose/L.)				Type of gland	Diagnosis and remarks
No.	Age	Initials	Sex	Wt. (Kg.)	24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.		
1	2½ mos.	W.S.	M	—	38				3.22	9.86				Macroglossia
2	8 mos.	H.M.	M	10.0	27	21			0.418	0.517				7 Days after herniorrhaphy (umbilical); slight respiratory infection during test
3	15 mos.	P.R.	F	9.5	35	33	29		0.252	0.387	0.506			5 Days after herniorrhaphy (umbilical)
4	18 mos.	C.P.	M	14.5	18	25			0.094	0.127				3 Days after pneumonia; afebrile
5	20 mos.	T.M.	F	11.3	32	34			0.068	0.288				3 Days after respiratory infection
6	21 mos.	P.M.	M	12.3	40				0.182					Phenylpyruvic oligophrenia
7	22 mos.	A.M.	M	11.3	26	27			0.225	0.510				None
8	2 yrs.	J.M.	F	12.7	30		36		0.206	0.522	0.810			5 Days after herniorrhaphy (umbilical)
9	2 yrs.	G.W.	F	8.8	19	13			0.054	0.176				Post-pneumonia; afebrile
10	2 yrs.	E.H.	F	12.0	29	33			0.106	0.160				Estropia
11	2 yrs.	E.G.	F	15.0	44	38			0.085	0.146				Keloid (old burn)
12	2½ yrs.	G.K.	F	11.3	26				0.104					Iron-deficiency anemia
13	3 yrs.	G.W.	M		39	30	30	30	0.028	0.061	0.097	0.127		2 Mos. after poliomyelitis
14	3 yrs.	D.P.	M	15.9					0.155	0.467				Old burn; 2 mos. after acute stage
15	4 yrs.	J.B.	F	19.7	40	34			0.068	0.154				Recurrent cystitis; afebrile
16	5 yrs.	A.B.	M	17.5	19	25	22		0.100	0.072	0.080			Seizures; etiology unknown
17	5 yrs.	R.C.	M	19.1	29	26	21	23	0.040	0.040	0.070	0.012		Psychogenic vomiting 1 wk. before
18	5 yrs.	A.M.	F	17.3	28	21			0.079	0.055				Syndactyly
19	5 yrs.	L.C.	F	23.1	30				0.019					None
20	6 yrs.	C.M.	F	22.5	24				0.005					Cystitis; afebrile
21	6 yrs.	C.T.	F	19.7	30				0.046					Myasthenia gravis
22	6 yrs.	D.S.	M	39.0	25				0.035					Obesity
23	7 yrs.	C.M.	M	20.5	30	24	25		0.047	0.052	0.087			10 Days after acute stage of meningitis
24	7 yrs.	J.R.	M	25.6	31	33	36		0.054	0.052	0.050			Laceration of finger
25	7 yrs.	J.W.	M	29.0	32	31			0.033	0.023	0.058			5 Days after acute stage, Cellulitis of finger
26	8 yrs.	W.B.	M	27.2	20	22	21		0.072	0.077	0.115			22 Days after acute stage, Cellulitis of thigh
27	8 yrs.	L.D.	M	18.6	32	36	33		0.042	0.036				Hookworm
28	8 yrs.	H.R.	F	23.1	36	36			0.088	0.253				7 Days after tooth extraction
29	8 yrs.	E.W.	F	28.6	19				0.016					Cerebral palsy
30	9 yrs.	G.S.	F	61.3	29				0.072					Obesity and slipped fem. epiphysis
31	10 yrs.	M.T.	M	20.9	30	33	28		0.046	0.045	0.028			Hookworm
32	12 yrs.	C.R.	M	52.7	34				0.061					None
33	12 yrs.	H.T.	M	41.8	42				0.028					Bronchial asthma
34	13 yrs.	A.C.	M	97.7	17				0.013					Obesity and slipped fem. epiphysis
35	13 yrs.	S.M.	F	82.0	26				0.015					Obesity
36	13 yrs.	L.M.	M		42	38			0.020	0.088				None

(*Continued on next page*)

TABLE 1—(*continued*)

Patient					Thyroidal I^{131} uptake (% dose)				Serum PBI^{131} (% dose/L.)				Type of gland	Diagnosis and remarks
No.	Age	Initials	Sex	Wt. (Kg.)	24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.		
37	14 yrs.	F.V.	F	61.0	28				0.016	0.022				None
38	14 yrs.	M.C.	F	69.0	18				0.013					Polycystic ovaries
39	14 yrs.	L.G.	F	41.0	32				0.028					None
40	14 yrs.	J.L.	F	56.0	25				0.017					Thyroid, 1–3 gr. daily for period 1 yr. to 10 wks. before test
41	14 yrs.	L.R.	F	52.0	33				0.062	0.066				None
42	14 yrs.	J.M.	F	52.7	23				0.036					Menometrorrhagia
43	14 yrs.	J.B.	M		46	48		46	0.046	0.114		0.218	Diffuse enlarg., 1½ ×normal	Inactive rheum. heart disease
44	14 yrs.	P.L.	M	55.0	31				0.034				Diffuse enlarg., 1½ ×normal	None
45	14 yrs.	J.H.	M	78.0	31				0.033	0.023			Afebrile rheum.	heart disease
46	15 yrs.	E.H.	F	50.0	19				0.034				Diffuse enlarg., 1½ ×normal	Amenorrhea 6 mos.
47	16 yrs.	E.Z.	F	59.0	42	40			0.020	0.023			Diffuse enlarg., 2 ×normal	Nonparalytic poliomyelitis (mild); afebrile 1 wk.
48	16 yrs.	D.P.	F	68.0	35				0.014					None
49	16 yrs.	C.S.	F	60.2	34				0.033					Adrenogenital syndrome
50	16 yrs.	J.M.	F	61.0	39				0.101					Tuberculous adenitis
51	16 yrs.	R.B.	M	54.5	42				0.015				Diffuse enlarg., 1½ ×normal	Thyroid, ½ gr. daily for period 5 mo. to 1 mo. before test None
52	16 yrs.	G.M.	M	59.0	31				0.019					Delayed puberty
53	16 yrs.	R.F.	M	53.4	28				0.014					None
54	17 yrs.	G.S.	F		40				0.062				Diffuse enlarg., 2 ×normal	PAS therapy (prolonged), 2 yrs. before test
55	17 yrs.	J.G.	F	52.2	25				0.011	0.020				Diabetes mellitus
56	17 yrs.	D.R.	F	62.0	32				0.029				Nodule, 2 cm. diam.	Thyroid nodule
57	18 yrs.	S.W.	F	54.5	37				0.030					? Adrenogenital syndrome
58	18 yrs.	N.A.	F	45.4	37				0.029					Turner's syndrome
59	18 yrs.	D.A.	M	56.3	50	52		52	0.022	0.033		0.100	Diffuse enlarg., 1½ ×normal	1 Wk. after acute stage of gastro-enteritis
60	18 yrs.	B.D.	F		34									None
61	18 yrs.	R.M.	F	57.2	26				0.039	0.028				None

tion phosphor attached to a #5819 photomultiplier tube. A lead filter of 1 mm. thickness was inserted in front of the crystal in order to absorb scattered radiation (this lead effectively absorbs 90 per cent scattered I^{131} gamma rays). The collimation of the scintillation counter was such that at the distances employed the tube "looked" at a field 6 inches in diameter with equal sensitivity; the isoresponse dropped to 10 per cent with a diameter of 7 inches. Thus errors due to extraneous body radiation were eliminated.

(b) *Serum protein-bound iodine^{131} determination.* The PBI^{131} determinations were made following the procedures outlined by Fields and Kaplan (12). A 5-ml. sample of plasma was placed in a plastic test tube. The protein fraction was precipitated by the addition of 1.5 ml. of 40 per cent trichloroacetic acid, and washed three times with 10 per cent

TABLE 2. I^{131} DATA ON CHILDREN WITH VARIOUS THYROID DISORDERS

Patient			Diagnosis	BMR (%)	Serum PBI (μg./100 ml.)	Thyroidal I^{131} uptake (% dose)		Serum PBI^{131} (% dose/L.)	
Age	Initials	Sex				24 hrs.	48 hrs.	24 hrs.	48 hrs.
4 mos.	E.C.	F	Cretin		1.0	3		0.26	
9 yrs.	R.S.	M	Hypothyroidism, mild			9		0.137	0.147
11 yrs.	B.T.	M	Hypothyroidism, hemachromatosis		0	7			
11 yrs.	L.S.	F	Cretin, retarded	−19	1.6	0		0.045	
12 yrs.	J.D.	M	Hypothyroidism	−28		15		0.144	0.332
9 yrs.	V.R.	F	Hyperthyroidism	+20		72	71	3.6	3.4
11 yrs.	H.D.	F	Hyperthyroidism	+38		99		0.45	
13 yrs.	V.McK.	F	Hyperthyroidism	+58		94	92	0.45	0.79
18 yrs.	B.E.	F	Hyperthyroidism	+54		95	85	0.93	0.96
16 yrs.	D.O.	M	Goiter since age 3	−26		83		0.009	
10 yrs.	M.D.	F	Thyroid carcinoma with cervical metast.			34		0.035	
12 yrs.	T.B.	M	Thyroid carcinoma, recurr.; previous surgery and irrad.			24		0.097	

trichloroacetic acid. All washings were discarded. The precipitate was counted in a scintillation well-counter for fifteen minutes. From the known efficiency of the counter (which was calculated daily), the dilution factor and the time counted, the net counts were converted to the percentage of the administered dose per liter of plasma (12).

(c) *Radiation safety.* The maximum permissible concentration of I^{131} in the total body is 4.15 μc. per Kg. (13). This is the dose which will deliver 50 milliroentgens-equivalent to total body tissue during the first twenty-four hours of exposure (14). Using this figure, it should be possible to give 100 μc. of I^{131} as a tracer dose to a child weighing 25 Kg.

The foregoing value does not consider the preferential concentration of the isotope in the thyroid gland. The tracer doses given to young children were about 25 μc. Assuming 30 per cent uptake by the gland, there would be about 7.5 μc. in an estimated 10-gram gland, or 0.75 μc. per gram of thyroid tissue. Using standard formulae (13) for calculating the dosage delivered to the thyroid gland from a beta- and gamma-emitting isotope, the gland of a normal child would receive about 90 equivalent roentgens per gram of tissue, assuming an adult biologic half-life. This amount of radiation is much

TABLE 3. I^{131} DATA ON NORMAL CHILDREN AND NORMAL AND HYPERTHYROID ADULTS

	No.	Thyroidal I^{131} uptake (% dose at 24 hrs.)		No.	Serum PBI^{131} (% dose/L. at 24 hrs.)		No.	Serum PBI^{131} (% dose/L. at 48 hrs.)		No.	Serum PBI^{131} (% dose/L./Kg.)	
		Range	Mean & s. d.		Range	Mean & s. d.		Range	Mean & s. d.		Range	Mean & s. d.
Euthyroid adults	64	16– 50	32.17± 7.26	64	0.002–0.077	0.0298±0.015	31	0.016–0.074	0.040±0.018	64	0.00003–0.00142	0.00044±0.00029
Hyperthyroid adults	62	34–100	76.11±14.84	62	0.081–2.90	0.423 ±0.499	22	0.151–2.86	0.641±0.85	62	0.0011–0.051**	0.0065 ±0.0054
Children; entire normal group	60	17– 50	31.10± 7.63*									
Age 1–4				13	0.028–0.252	0.125 ±0.071	11	0.061–0.522	0.272±0.169	12	.0035–.0265	0.0111 ±0.0069
Age 5–9				15	0.005–0.088	0.049 ±0.028	9	0.023–0.253	0.073±0.069	15	0.00022–0.0057	0.0022 ±0.0015
Age 10–18				28	0.011–0.101	0.031 ±0.020	8	0.020–0.066	0.032±0.016	27	0.00013–0.00165	0.00059±0.00048

* At 48 hours for 24 children (range 13–52 per cent; mean, 31.4±8.80).

** 0.051 level omitted in calculating the mean and s.d.

below the level at which a possible association between external x-radiation of the neck and upper chest and the production of thyroid carcinoma has been intimated (15). This association has been questioned (16). In addition, since 1939 no carcinogenic effect due to radioiodine has thus far been reported in the adult receiving up to 50,000 equivalent roentgens per gram of tissue in the treatment of hyperthyroidism and of euthyroid cardiac patients.

RESULTS

The results of this study are tabulated in Tables 1 and 3.

Thyroidal uptake

Uptakes at twenty-four hours for 60 of the 61 normal children ranged from 17 to 50 per cent, with a mean of 31.1 ±7.63 (S.D.) (Fig. 1 and Table 3). This range and mean did not differ from those of 64 euthyroid adults. There did not appear to be any difference between boys and girls, nor was there any variation with age. The uptake measurement after twenty-four hours did not indicate a definite pattern of either rise or fall. In fact, the range and mean for forty-eight hour uptakes did not differ statistically from the 24-hour values. No correlation between uptake and body weight was noted. Uptake data on 5 hypothyroid children showed a range of 3 to 15 per cent at twenty-four hours, whereas in 4 hyperthyroid children the range was from 72 to 99 per cent. Therefore, in this series, there was no

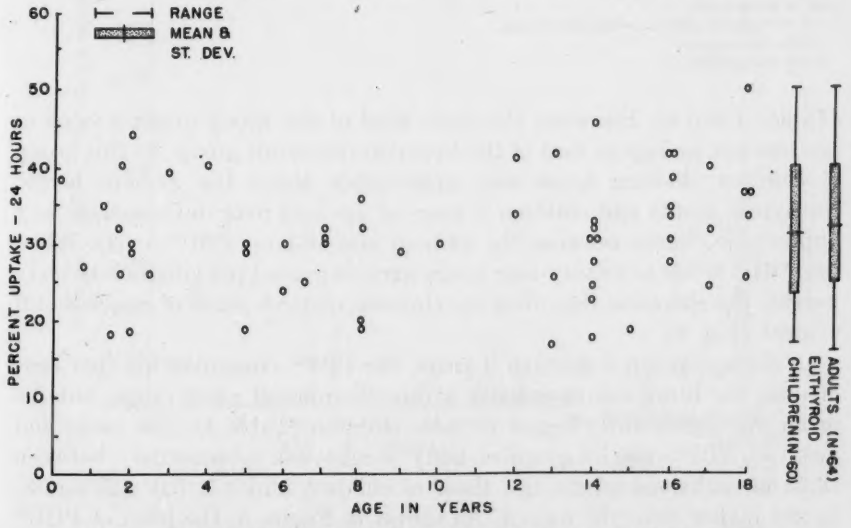

FIG. 1. Thyroidal I^{131} uptake studies at twenty-four hours in 60 normal children up to age 18 compared with data on 64 euthyroid adults.

overlap between thyroidal I^{131} uptake values for normal children and for those with thyroid dysfunction.

Serum levels of PBI^{131}

The difference in PBI^{131} levels in terms of per cent of administered dose per liter of plasma in the different age groups of normal children proved to be highly significant. Since there were only 2 children below the age of 1 year, they were excluded from the analysis shown in Table 4. In the group from 1 through 4 years of age the PBI^{131} mean level was elevated significantly above the mean for older children and for euthyroid adults

TABLE 4. STATISTICAL ANALYSIS OF PBI^{131} DATA IN CHILDREN AND ADULTS

Variable	Compared groups						u^*	P (2-tailed)
	Group 1	No. in group	Mean	Group 2	No. in group	Mean		
PBI^{131} (% dose/L./24 hrs.)	1 through 4	13	.1251	5 through 9	15	.0499	30.5	<.002
	1 through 4	13	.1251	10 through 18	28	.0312	23.0	<.0001
	1 through 4	13	.1251	E.A.†	64	.0289	36.0	<.000001
	1 through 4	13	.1251	H.A.†	62	.4235	139.0	<.001
	5 through 9	15	.0499	E.A.	64	.0289	244.5	<.01
	10 through 18	28	.0312	E.A.	64	.0289	N.C.‡	N.S.§
PBI^{131} (% dose/L./Kg./24 hrs.)	5 through 9	15	.00216	E.A.	64	.00044	77.5	.0001

* This was preferred over the *t* test because the variances of samples were very different statistically. For more detailed discussion of the *u* test, *see* reference (31).

† E.A. = Euthyroid adult; H.A. = Hyperthyroid adult.

‡ N.C. = Not computed.

§ N.S. = Not significant.

(Tables 3 and 4). However, the mean level of this group under 5 years of age was not as high as that of the hyperthyroid adult group. In this group of children, 48-hour levels rose appreciably above the 24-hour levels. Euthyroid adults and children 5 years of age and over did not show any appreciable change between the 24-hour and 48-hour PBI^{131} levels. When the PBI^{131} levels at twenty-four hours were estimated per kilogram of body weight, the elevation described for children under 5 years of age was still evident (Fig. 2).

In the age group 5 through 9 years, the PBI^{131} concentration (per cent of dose per liter) was essentially within the normal adult range, but the mean was significantly higher in these children (Table 4). The range and mean of PBI^{131} per kilogram of body weight was intermediary between those of euthyroid adults and those of children under 5, but still significantly higher than the former. As shown in Figure 3, the level of PBI^{131} per kilogram was significantly elevated, until a body weight of 25 to 30 kilograms was reached (7–9 years of age). Figure 3 also shows the relation-

ship between oxygen consumption (calories per kilogram of body weight) and increasing weight for normal children (17). There is increased oxygen consumption up to approximately 30 kilograms, paralleling the elevation of the level of PBI^{131} per kilogram.

The PBI^{131} levels of children 10 through 18 years old did not differ in any respect from the values in euthyroid adults.

Two children with hypothyroidism (R. S. and J. D., Table 2) had elevated PBI^{131} values. We have noted elevated PBI^{131} levels in patients with Hashimoto's thyroiditis (18). The etiology of hypothyroidism in these 2 children, therefore, may have been some form of thyroiditis.

Two supposedly normal children who had incurred recent cerebral concussions were excluded from this study because of 24-hour thyroidal I^{131} uptake values of 8 and 14 per cent. No explanation can be given for this finding at present.

DISCUSSION

The data accumulated in this study indicate a state of thyroidal "hyperactivity" through the age of 4 years and possibly as late as age 9. This

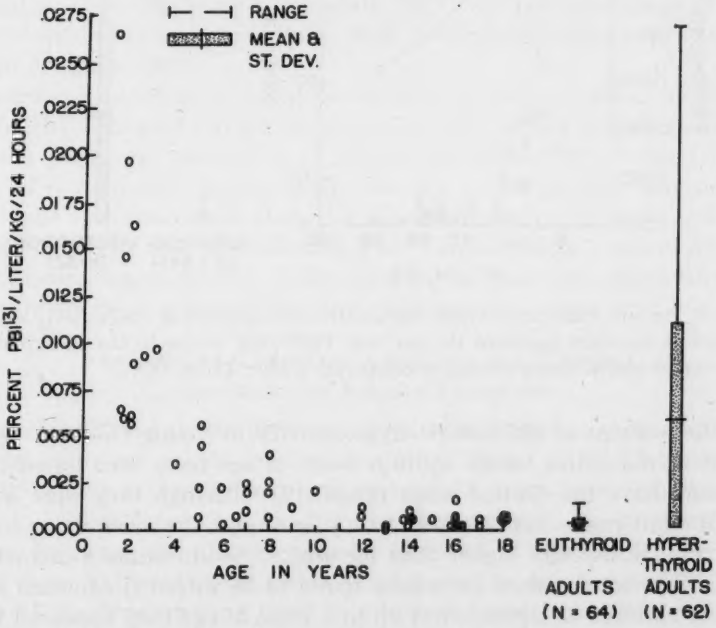

FIG. 2. Per cent PBI^{131} per kilogram of body weight at various ages in children compared with that in euthyroid and hyperthyroid adults. The bars at the right represent the per cent PBI^{131}/Kg. values in the euthyroid and hyperthyroid adults.

physiologic hyperactivity, as shown by PBI^{131} values, is evident through the fourth year of life and thereafter decreases gradually, so that by the tenth year the adult euthyroid state has been attained. Although only 2 infants under 1 year of age were studied, it is worthwhile to note that their PBI^{131} levels were the highest recorded for all normal children.

Serum protein-bound iodine determinations, thyroid weight and microscopic findings in human subjects, as well as data on young animals, sup-

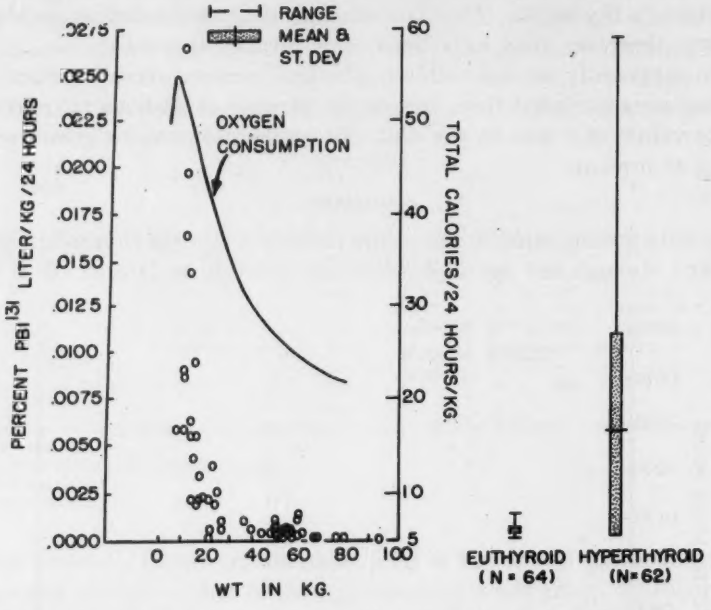

FIG. 3. Per cent PBI^{131} and oxygen consumption per kilogram at various body weights. The bars at the right represent the per cent PBI^{131}/Kg. values in the euthyroid and hyperthyroid adults. (Data on oxygen consumption after Talbot (17).)

port the concept of physiologic hyperactivity in young children. Serum protein-bound iodine values up to 6 weeks of age have been found to be elevated above the normal adult range (19); although they were within normal adult range from 6 weeks to 1 year of age, the mean value for infants was statistically higher than for adults. Serum butanol-extractable iodine (thyroxine) values have been found to be within the normal adult range in children as a group, but up to 7 years of age they appeared to be higher than in adults (20). PBI levels studied up to the age of 18 have been reported to be within the normal adult range, but to be decreased with increase in age; the lowest values were noted during early adolescence (21).

These data plus those of our own studies fail to support the concept of increased thyroid activity during the prepubertal growth spurt.

The ratio of thyroid weight to total body weight has been reported as much higher in infants (1:700–1,000) than in adults (1:2,000–2,500) (22).

Cooper in 1925 (23) reviewed the histologic changes of the thyroid at various ages. She interpreted the histologic appearance of the thyroid through childhood and adolescence as being compatible with hyperactivity. In fact, the illustrated sections in older children showed a structure similar to the irregularity of acini, lymphocytic infiltrations, and cuboidal to tall-columnar epithelium found in adult thyrotoxicosis.

Using the thiouracil goiter-prevention assay, the thyroidal secretion rate has been found to be two to four times greater in growing than in mature mice (24). Similar data were obtained in rabbits (25). The "I^{131} secretion rate of the thyroid" of young rats has been reported to be more rapid than that of old rats of equal body weight (26).

The elevation of the serum PBI^{131} level in early childhood may possibly be due to decreased body fluid spaces per unit of body weight as compared to the adult. This seems unlikely because plasma volume per unit of body weight is about the same as in adults (27), and the percentage of body weight made up by extracellular fluid is increased slightly above that found in adults (28).

Increased metabolic activity during infancy and childhood, as demonstrated by increased oxygen consumption (17), seems to indicate an increased peripheral utilization of thyroid hormone. The elevated PBI^{131} level, if it represents an increased hormonal secretion rate, may demonstrate the response of the thyroid to this increased peripheral need. The possibility exists that the elevated PBI^{131} level reflects a decreased thyroidal iodine space. Studies of thyroidal iodine space and thyroidal secretion rate are in progress now to clarify this point.

COMMENT ON PERTINENT RADIOIODINE STUDIES REPORTED ON NORMAL CHILDREN

Listed in Table 5 are the pertinent radioiodine studies on normal children as reported from various areas of the United States (1–8). Thyroidal I^{131} uptake values at twenty-four and forty-eight hours reported from the East and West Coasts are much lower than our data. In previously reported data from the Chicago area (5) there was a mean of 21.3 per cent ± 6.7 (S.D.) at twenty-four hours as compared to our mean of 31.1 per cent ± 7.63. Locality may have an effect on normal values in children, but the following factors may also have a bearing on the differences between the reported values and our own: 1) the radiochemical purity of the radioiodine administered, and 2) the intake of cod liver oil. The

TABLE 5. PERTINENT RADIOIODINE DATA ON NORMAL CHILDREN, AS REPORTED IN THE LITERATURE

Authors (locality)	Number tested	Age group	Test	Counting equipment	Dose of I^{131} (μc.)	Results
Quimby & McCune (ref. 7, 1947) (New York)	54	3 wks. to 14 yrs.	Thyroidal 48-hr. uptake	G-M	20–40	Uptake, 7–20%; mean 12%
Talbot (ref. 6, 1955) (Boston)	Not stated	Not stated	Thyroidal 24-hr. uptake	Counter against neck	1–3 (to 5 yrs.) 3–8 (to 15 yrs.)	Uptake (up to 2 yrs.), 12.5% Uptake (after 2 yrs.), 10–40%
Friedman (ref. 5, 1955) (Chicago)	68	1½–16 yrs.	Thyroidal 24-hr. uptake	G-M, 20 cm. dist. from neck	10–20	Uptake, 9–38%; mean 21.3 ± 6.7
Reilly & Bayer (ref. 1, 1952) (San Francisco)	25	9 mos. to 15 yrs.	Thyroidal 24-hr. to 96-hr. uptakes	G-M, RAE stand., 4 cm. dist. from neck	5–10	Uptake, 8.7–29.8%; avge. 16.9% (usually at 24 hrs.; some as late as 96 hrs.)
Pickering & Miller (ref. 2, 1953) (San Francisco)	4	1–10 yrs.	Thyroidal 24-hr. uptake		15–40	Uptake, 12–20%
Van Middlesworth (ref. 3, 1954) (Memphis)	7	Newborn; 2–3 days	Thyroidal 14-hr. uptake	Scint., against neck	1	Uptake, 46–97%; 6 of 7 above 61%
Martmer (ref. 4, 1955) (Detroit)	16	Premature	Thyroidal 24-hr. uptake	G-M, 5 cm. dist. from neck. Scint., 13 cm. dist. from neck	5	Uptake, 28–52% (euthyroid adult, 30–45%)
Reilly, Scott, Winters & Helwig (ref. 8, 1955) (San Francisco)	14	Not stated	Serum PBI^{131} level at intervals to 110 hrs. (counts/sec. % per 70 Kg.)		10–30	PBI^{131} as early as 6–8 hrs.—earlier and in greater amounts than in euthyroid adult.

radioiodine administered in the study by Quimby and McCune (7), produced in the cyclotron, was not tested for radiochemical purity (29). We are unable to judge whether this affected the values reported. Cod liver oil has been used extensively for many years as a vitamin supplement for infants and children. This fish oil has a very high iodine content (30) and may have been given to children used as subjects in some of the early studies, possibly accounting for the lowering of the thyroidal uptakes.

It appears that normal radioiodine values in children should be re-evaluated in different localities of the United States, using consistent counting methods, and taking into consideration that iodide-containing vitamin supplements should be discontinued for an appropriate time before testing. It may also be feasible for the Atomic Energy Commission to circulate a phantom child's thyroid gland corresponding to the phantom adult gland recently circulated by them. This could be used for standardization purposes in laboratories interested in such studies.

SUMMARY

Radioiodine studies were performed on 73 children between the ages of 2½ months and 18 years; 61 of the subjects were considered normal so far as thyroid function was concerned, and 12 had various thyroid disorders.

Thyroidal 24-hour and 48-hour I^{131} uptake values for the normal group were within the normal adult range and were not affected by body weight or sex. Up to the age of 4 years, PBI^{131} values at twenty-four and forty-eight hours were significantly elevated, as compared with the adult. Although the values in the 5 to 9 year group were within the normal adult range, the mean value was significantly elevated. The PBI^{131} data may indicate a state of "hyperactivity" of the thyroid gland in children up to the age of 4 years, and possibly longer, when compared to the euthyroid adult. Studies are in progress to elucidate this finding.

Values obtained for thyroidal I^{131} uptakes in this study have been compared with previously reported values, and the differences discussed.

Acknowledgment

The authors are indebted to Dr. Helen A. Heath for the statistical evaluation of the data presented.

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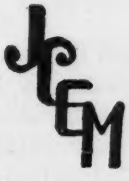

EFFECT OF ANESTHESIA AND THYROID SURGERY ON THE PLASMA LEVEL OF PBI^{131}*

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MANIPULATION of the thyroid gland during surgery, with the consequent release into the circulation of significant amounts of preformed thyroid hormone, has been suggested as a possible factor in the genesis of postoperative "storm" (1–4). In addition, it has been postulated that a definite correlation exists between the magnitude of this phenomenon and the severity of the postoperative reaction (1, 5). Reports of data obtained prior to the use of radioactive iodide in the assessment of thyroid function are conflicting in regard to changes in serum protein-bound iodine levels under these circumstances (1, 5–9). Measurements of the renal excretion of stable iodide have demonstrated a significant increase immediately following both thyroid (7, 10) and non-thyroid surgery (11, 12). That elevations of protein-bound iodine concentration can occur in response to therapeutic doses of radioiodide (13) and irradiation of the thyroid gland with radium or x-rays (5, 14) seems well documented.

The present study was designed to evaluate changes in the plasma level of PBI^{131} coincident with anesthesia and thyroid surgery by assaying the activity of this fraction, labelled *in vivo* by the prior administration of radioiodide. An increase of this moiety should reflect the release of tagged substances from the thyroid gland and serve as a prompt indicator of the sequential changes that might occur in response to the stress or metabolic alterations incident to anesthesia, surgery and/or manipulation of the thyroid gland. It is necessary to assume, however, that changes in the volume of distribution and the rate of degradation of the extrathyroidal

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organic iodine pool are relatively insignificant during the short period of observation (15).

METHOD

Eleven euthyroid individuals with nontoxic solitary or multinodular goiters, ranging in age from 35 to 73 years, were given carrier-free radioiodide by mouth forty-eight to seventy-two hours prior to thyroid surgery. The "tracer" dose varied from 32 to 1,500 microcuries. None of these subjects had received any substance known to affect the uptake, degree of binding, or release of iodide or iodinated amino acids by the thyroid gland.

The first peripheral venous blood sample was withdrawn from ten to two hundred and ten minutes before anesthesia, and served as the base line of activity in the total plasma, and in that fraction designated as PBI^{131}. Nitrous oxide and ether anesthesia with endotracheal intubation was used in all but 2 patients; these 2 were given superficial cervical blocks. A second blood specimen was obtained in most instances immediately upon completion of anesthesia. The third sample was drawn from thirty to one hundred and twenty minutes following the completion of thyroid surgery (unilateral or bilateral subtotal lobectomy). No blood transfusions were administered, but all subjects received infusions of 5 per cent glucose in water in amounts ranging from 250 to 1,000 ml. The time for the actual operative procedure varied from thirty minutes to three hours.

The radioactivity of the plasma samples was determined in 4-ml. aliquots by assay in a well-type scintillation detector (previously calibrated with I^{131}) for periods of time sufficient to obtain statistically significant counts with an expected standard error of less than 10 per cent. The PBI^{131} determinations were performed by precipitation with trichloracetic acid, of the same plasma sample utilized for the measurement of total activity, according to the technic described by Silver (16). All counts were corrected for background and for physical decay to the time of administration, and the results expressed as a percentage of the administered dose of I^{131} per liter of plasma.

RESULTS

The net effects of anesthesia and thyroid surgery on the levels of plasma PBI^{131} are illustrated in Figure 1. It is apparent that none of the subjects demonstrated any remarkable fluctuation in protein-bound plasma radioactivity during the period of study, and no definite trend is evident. The curves closely resemble those regularly seen in euthyroid persons forty-eight to seventy-two hours subsequent to the administration of radioiodide in tracer amounts (17, 18). The moderate rise of the PBI^{131} values depicted graphically in 3 of the 11 patients (B.M., E.S. and J.V.) is not considered to be statistically significant, since all the measurements agree within two standard deviations. In addition, there was no consistent fluctuation in the unbound fraction of I^{131} (Fig. 2), which exceeded the limits of the inherent counting error. An increase in this latter moiety might have been expected with accelerated peripheral utilization of thyroid hormone had (a) urinary excretion or salivary secretion, (b) re-utilization by the thyroid gland, and (c) increase in the volume of distribution been of relatively low magnitude during the period of observation. The data

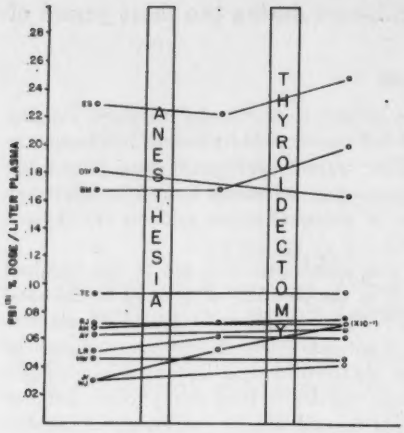

FIG. 1. PBI^{131} activity (% dose/liter plasma) of peripheral venous samples in 11 euthyroid individuals before anesthesia, immediately following completion of anesthesia, and at the termination of thyroid surgery.

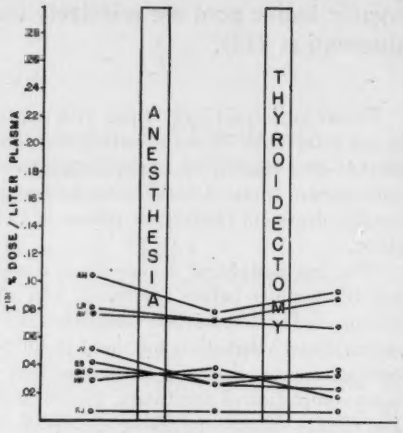

FIG. 2. Inorganic I^{131} activity (% dose /liter plasma) of peripheral venous samples in 11 euthyroid individuals before anesthesia, immediately following completion of anesthesia, and at the termination of thyroid surgery.

presented in Figures 1 and 2 have been recalculated on the unlikely assumption that all of the intravenous fluids infused during surgery might have been retained in the intravascular compartment until the final blood sample was withdrawn. The recalculated data still do not show any significant deviation from the preanesthetic values. All of these patients experienced an uneventful postoperative course.

DISCUSSION

There have been relatively few studies of the plasma levels of organic iodine in the *immediate* post-thyroidectomy period. Measurable increases of circulating thyroglobulin have been detected immunologically in thyroid vein blood in patients with toxic and nontoxic goiters during and immediately following thyroid surgery (19), suggesting that the operative procedure itself caused extrusion of this material into the general circulation. It is of interest that there was no correlation between the severity of the postoperative reaction and the serum titer of thyroglobulin in any of these subjects; the possibility that extrusion of other physiologically active iodinated substances might have played an important role in the development of thyrotoxic crises could not be excluded, however. The protein-bound iodine determined by the present chemical and isotopic methods includes thyroxine and the other iodinated amino acids synthesized by the

thyroid, in addition to thyroglobulin. Elevations in concentration of this protein-bound fraction have been reported following thyroid surgery (8, 9). The postoperative samples in these instances, however, were not obtained in the immediate period after the termination of the operation, or else the patients had received large doses of Lugol's solution as preparation for thyroidectomy (1). Delayed rises in the concentration of the butanol-extractable I^{131} moiety have also been recorded in several cases of nonthyroid surgery (20).

In our study, the failure to detect a significant rise in the level of the plasma PBI^{131} fraction immediately following general anesthesia and thyroid surgery suggests, but does not conclusively prove, that these physiologic and mechanical stimuli were not of sufficient magnitude to cause the rapid appearance of large increments of hormonal iodine in the plasma in this group of 11 euthyroid patients. Data derived solely from plasma PBI^{131} levels are difficult to evaluate in terms of the absolute output of physiologically active substances by the thyroid gland, since the degree of homogeneity and the specific activity of the thyroidal pool of organic iodine are variable factors. Stable levels of plasma protein-bound iodine could have conceivably occurred, however, during the brief intervals included in this study if the rate of hormonal degradation had accelerated at a rate equal to that of the increase in the release of iodinated organic substances from the thyroid gland. In addition, expansion of the volume of the extrathyroidal space of distribution would tend to mask any influx of endogenously labelled protein-bound iodine. This contingency seems unlikely in view of the fact that previous studies have indicated that there is a lag of at least several hours before the major portion of intravenously administered tagged thyroxine becomes distributed throughout the extrathyroidal compartment (15, 21).

Plasma specimens were not sampled any later than the immediate post-thyroidectomy period, which did not exceed one hundred and twenty minutes. It is possible that the delayed elevations in the level of protein-bound iodine reported by others (8, 9, 20) result from postoperative metabolic readjustments involving complex neuroendocrine mechanisms, and might have been found in our patients had they been followed longer.

It is hazardous to extend these observations in individuals with nontoxic goiters to hyperthyroid patients. It should be noted, however, that neither the total iodine content nor the concentrations of the various iodinated compounds in the thyroid glands of untreated thyrotoxic patients differ significantly from those of euthyroid control subjects (22). Also, there is no unique histologic variation in the toxic thyroid that has not been demonstrated in completely nontoxic glands (3). On the basis of these observations and the data reported herein, it seems unlikely that

the syndrome comprising thyroid "storm" can be accounted for by the mechanical extrusion of preformed hormone into the systemic circulation during manipulation of the thyroid gland.

SUMMARY

1. No detectable change in the plasma PBI^{131} concentration was found in 11 euthyroid patients with solitary or multinodular goiters immediately following anesthesia or thyroid surgery.

2. The possible implications of these observations with respect to post-thyroidectomy reactions are discussed.

Acknowledgments

The author is indebted to Mr. Robert Gerson for advice, to Miss Sandra Betz for technical assistance, and to the surgical service of Dr. I. S. Ravdin and the anesthesiology staff of Dr. Robert Dripps for their cooperation.

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EFFECTS OF AN ORGANIC IODINE COMPOUND (PRIODAX) ON TESTS OF THYROID FUNCTION*

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VARIOUS organic iodine compounds used in radiology affect the succeeding tests of thyroid function. Certain iodine-containing compounds become associated with proteins in the serum (1) and are measured as protein-bound iodine. The serum protein-bound iodine (PBI), considered normally to consist predominantly of thyroxine, is therefore artefactually elevated. Some of the organic iodine compound does not associate with protein and the total iodine is raised, therefore, by an elevation in PBI, unbound organic iodine compound and iodide. The uptake by the thyroid of radioactive iodine may be reduced below normal. It has been assumed that this is due to a release from the organic iodine molecule of inorganic iodide—sometimes slowly over a long period of time. The development of myxedema following administration of organic iodine has been reported (2).

The comparison of total iodine with PBI often indicates contamination with inorganic iodide or with organic iodine compounds unassociated with protein. Butanol does not extract the protein-iodine complex which is formed when iodide levels have been high for some time, and serves to differentiate this artefactual elevation of PBI. Certain organic iodine compounds associated with protein, however, are soluble in butanol and thus both the PBI and BEI levels will be misleadingly elevated (3).

A study of organic iodine compounds in animals and human beings is presented.

MATERIAL AND METHODS

Priodax, [β-(4-hydroxy-3,5-diiodophenyl)-α-phenyl proprionic acid] [iodoalphionic acid], labelled with radioactive iodine was prepared by distillation of I_2^{131} into a flask containing β-(4-hydroxyphenyl)-α-phenyl proprionic acid, as described by Taurog for

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† Damon Runyan Memorial Fund Fellow.

the preparation of labelled thyroxine (4). The material was chromatographed on No. 1 Whatman paper in an ascending system of butanol-acetic acid-water (75:10:15) and the radioactive Priodax was eluted with 95 per cent ethanol. Priodax was similarly chromatographed and identified by applying a mixture of arsenious acid and ceric ammonium sulfate (5); the findings coincided with those for radioactive Priodax (R_f 0.92).

Radioactive Priodax (30–240 μc.; about 0.5–2.0 mg.) was given by stomach tube to rats and guinea pigs. Serum, feces and urine were chromatographed and whole body counts were made daily. Similar determinations were made upon the human being, positioned for whole body counting, in an arc of 94 cm. radius around a scintillation counter. β-Glucuronidase was added to urine samples buffered at pH 5 and incubated at 37° C. for eighteen hours. Then aliquots were chromatographed.

Telepaque [β-(3-amino-2,4,6-triiodophenyl)-α-ethyl proprionic acid] [iopanoic acid] was given to rats for one day by mouth and the thyroidal uptake of I^{131} was determined seven and thirteen days later. Propylthiouracil (0.03 per cent) was added to the diets of rats and thyroidal weights were determined ten days later in a group on the normal diet, in a group with Telepaque added, and in a group with an equivalent amount of inorganic iodide added.

The records for the past two years of patients having had cholecystograms preceding the determination of radioactive iodine uptake were reviewed. Several hyperthyroid and euthyroid patients were given Telepaque and measurements made, at intervals, of the thyroidal uptakes of radioactive iodine and of the serum PBI levels.

RESULTS

Animals

The half-life of radioactive Priodax in the rat and guinea pig was as short as five hours for the first day, and thereafter was about two to four days (Figs. 1 and 2). Some Priodax was deiodinated and chromatograms indicated radioactive iodide in the blood and urine. Enzymatic hydrolysis of the thyroid with pancreatin indicated the formation of radioactive iodinated tyrosines and thyroxine. Most of the radioactivity of the urine, however, was associated with Priodax and a compound which, upon incubation with β-glucuronidase, was converted into Priodax (Fig. 3). The feces also contained Priodax. The total radioactivity ten days after the ingestion of radioactive Priodax was higher in the thyroid, liver and bowel than in other organs, although the concentration was greater in the thyroid, pectoral muscle and kidney. A control rat was given an amount of radioactive iodide equivalent to the radioactivity of Priodax given another rat. In the first twenty-four hours the urine of the rat given Priodax contained much more total radioactivity than did the urine of the control rat. The radioactivity of urine from the rat given Priodax was in the form of Priodax glucuronide, and in smaller amounts, Priodax and iodide. The amount of iodide was less than that in the urine of the control rat. The radioactive compounds present in the thyroids were the same, but in much greater amounts in the control rat (Fig. 4).

The effect of an organic iodine compound, Telepaque, upon the uptake

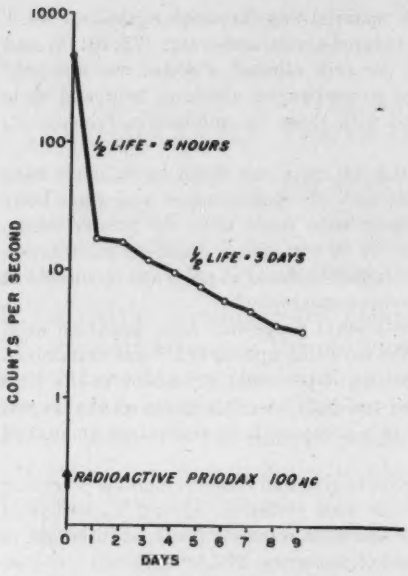

FIG. 1. Rat whole body counts per second after oral ingestion of 100 μc. of radioactive iodoalphionic acid (Priodax). The two distinct slopes indicate an initial rapid excretion which slows after the first day. Counts are plotted on a semilog scale. (Upon subtraction of the second component from the first, there is no significant change in the value for the half-life.)

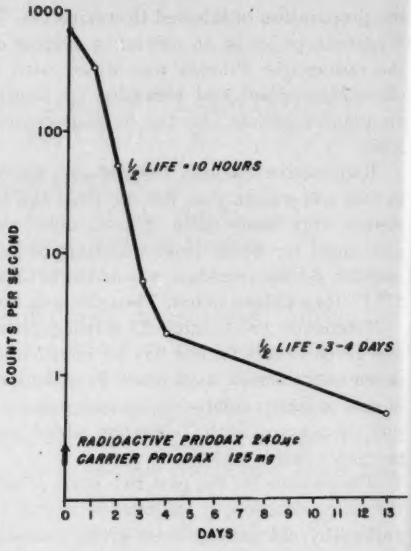

FIG. 2. Rat whole body counts per second after oral ingestion of 240 μc. of radioactive iodoalphionic acid (Priodax) and 125 mg. of carrier Priodax. The early rapid excretory phase lasts longer with carrier Priodax than without it. Counts are plotted on a semilog scale.

of radioactive iodine in rats was determined. One week after feeding 3 mg. of Telepaque to rats, the thyroidal uptake of I^{131} was diminished (68 per

TABLE 1. THE EFFECT OF IOPANOIC ACID (TELEPAQUE) AND AN EQUIVALENT AMOUNT OF INORGANIC IODIDE ON THE UPTAKE OF RADIOACTIVE IODINE BY THE RAT THYROID

Number of rats	Mink Chow diet	Inorg. iodide (1.92 mg. I^-/10 Gm. diet for 1 day)	Telepaque (3 mg. per 10 Gm. diet for 1 day)	Per cent of control value at 7 days	Per cent of control value at 13 days
3 and 4*	+	−	−	100	100
3 and 4	+	+	−	58	87
3 and 4	+	−	+	68	115

* A different group of rats for the two-week experiment.

cent of normal) but was approximately the same as that of a group of controls fed an equivalent amount of inorganic iodide. Thirteen days after feeding 3 mg. of Telepaque to rats the thyroidal uptake of I^{131} was slightly (115 per cent) above normal, and somewhat more (135 per cent) above that in a control group receiving an equivalent amount of inorganic iodide (Table 1). The addition of Telepaque to the diets of rats did not prevent propylthiouracil-induced goiters (Table 2).

Human subjects

Radioactive Priodax (300 μc.) with carrier Priodax (3 Gm.) was taken by mouth by a human subject. Whole body counts were made at frequent intervals to determine the half-life of the radioactive Priodax. Aliquots of 24-hour urine and stool collections were counted and chromatographed. Serum samples were also counted, chromatographed, and total iodine and PBI determinations were made. The half-life of radioactive Priodax in the human being was approximately twelve hours during the first three days, though a later slower phase of excretion may have been missed (Fig. 5). The excretory products detected in the urine were a small amount of iodide and a large amount of a compound which reacted with β-glucuronidase to yield Priodax. The radioactivity of the urine accounted for 60 per cent of the 300 μc. ingested, and the feces for approximately 4 per cent in the first twenty-four

FIG. 3. Ascending chromatograms in butanol-acetic acid-water (75:10–15) of an aliquot from a 24-hour urine of a rat given radioactive iodoalphionic acid (Priodax). The strip on the right is a more dilute aliquot of the same urine treated with β-glucuronidase for eighteen hours at 37° C., and indicates disappearance of Priodax glucuronide.

TABLE 2. THE EFFECT OF IOPANOIC ACID (TELEPAQUE) AND AN EQUIVALENT AMOUNT OF INORGANIC IODIDE ON PROPYLTHIOURACIL-INDUCED GOITERS IN THE RAT

Number of rats	Low I^- diet (3 μg./10 Gm. diet)	Propyl-thiouracil (0.03%)	Potassium iodide (83 μg./10 Gm. diet)	Telepaque (100 μg./10 Gm. diet)	Average wt. of thyroid (mg.)
4	+	−	−	−	9.2
4	+	+	−	−	24.8
4	+	+	+	−	20.5
4	+	+	−	+	23.7

hours. During the second twenty-four hours the urine contained 10 per cent and the feces about 3 per cent of the original dose. Chromatographic analysis of the feces indicated the presence of radioactive Priodax on the first day only. By the ninth day the radioactivity of the urine was not above background. Chromatography of a sample of serum taken at twenty-four hours indicated a trace of radioactivity with the same R_f as Priodax glucuronide, but no other radioactive material. One day following the ingestion of Priodax (3 Gm.) the total iodine level of the serum was 2,850 μg. per 100 ml. On the fourth day the level of total iodine was 176 μg. and of PBI, 80 μg. per 100 ml., and the levels continued as follows: on the sixth day, total iodine 80 μg. and PBI 48 μg.; on the thirteenth day, total iodine 34 μg. and PBI 32.4 μg.; on the nineteenth day, total iodine 24.3 μg. and PBI 21.7 μg. and on the thirty-seventh day, total iodine 14.7 μg. and PBI 10.2 μg. per 100 ml. Radioactivity in the thyroid region was never greater than 150–160 per cent of that in the thigh. The gallbladder region four hours after the ingestion of Priodax was eight times as radioactive as the thigh; at twenty-four hours the radioactivity was double that in the thigh, and on the third day it was equal to that in the thigh.

A woman with thyrotoxicosis and a thyroidal radioactive iodine uptake of 74 per cent was given 3 Gm. of Telepaque. There was no uptake of a tracer dose given three days later but there was a 91 per cent uptake of a tracer dose given seven days after the Telepaque (Fig. 6) Myxedema developed four months later during propylthiouracil therapy, at which time serum total iodine and PBI levels were each 2.1 μg. per 100 ml. Another patient with thyrotoxicosis and a radioactive iodine uptake of 88 per cent was given 3 Gm. of Telepaque; two days and four days later there was no uptake of a tracer dose, but six days later the uptake was 43 per cent (Fig. 7). The PBI and total iodine levels at this time were each over 32 μg.

FIG. 4. Strips A and D are chromatograms of urine and hydrolyzed thyroid from a rat given radioactive iodoalphionic acid (Priodax). Strips B and C are chromatograms of urine and hydrolyzed thyroid from a rat given radioactive iodine. Strip C indicates iodide, monoiodotyrosine (MIT), diiodotyrosine (DIT), and thyroxine (TX) in the rat given radioactive iodine. Strip D shows these same compounds in much smaller amounts. The urine of the rat receiving radioactive iodine contains only iodide (Strip B) and much less total radioactivity than the urine of the rat given Priodax (Strip A).

per 100 ml. A third patient with thyrotoxicosis and a radioactive iodine uptake of 63 per cent had no uptake of a tracer dose five days after 3 Gm. of Telepaque. Unfortunately, this patient could not be followed any longer. A euthyroid patient, who had had a cholecystectomy and thyroidal uptake of 25 per cent was given Cholographin (N,N-adipyl-bis(3-amino-2,4,6-

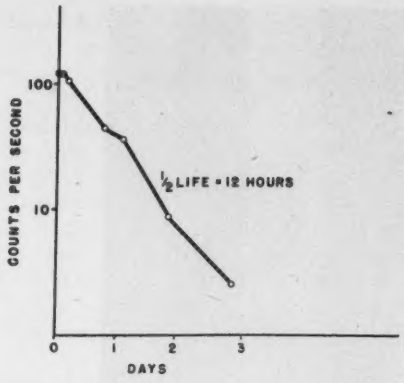

FIG. 5. Radioactive iodoalphionic acid in the form of Priodax (300 μc.) and carrier Priodax (3 Gm.) were taken by a human subject orally and the whole body counted at intervals. Counts per second are plotted on a semilog scale.

triiodobenzoic acid). Three days later the radioactive iodine uptake was 11 per cent and six days later it was 16 per cent. Two weeks later the PBI and total iodine levels were over 32 μg. per 100 ml. Telepaque was given when the uptake was 18 per cent; seven days later the uptake was 7 per cent, and thirteen days later it was 12 per cent. At this time the total iodine and PBI levels were over 32 μg. per 100 ml.

The short half-life of radioactive Priodax in the human subject and the rapid return to high levels of thyroidal radioactive iodine uptake following administration of Telepaque in a few hyperthyroid patients suggested the possibility that the generally accepted impression of the long influence of

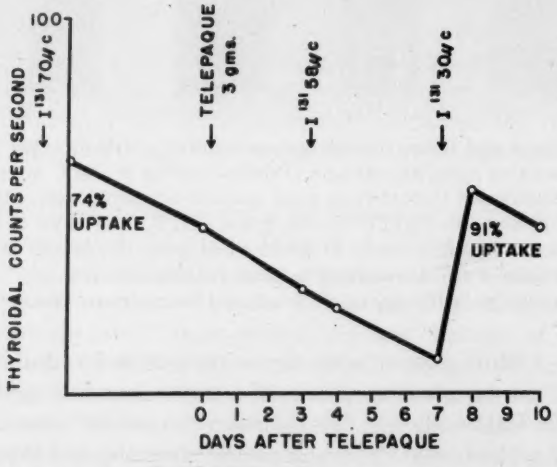

FIG. 6. The effect of iopanoic acid (Telepaque) upon the uptake of radioactive iodine in a patient with thyrotoxicosis. Counts per second are plotted on a semilog scale.

cholecystograms on radioactive iodine uptakes might be exaggerated. It was therefore thought to be of interest to tabulate the radioactive iodine uptakes at twenty-four hours after cholecystograms. Hospital records of 102 patients who had received tracer doses of radioactive iodine within one year of a cholecystogram were examined. A review of the data on 89 euthyroid patients indicated a great variation in the uptakes of radioactive iodine following cholecystograms (Fig. 8). However, it was noted that of 24 patients who were given radioactive iodine within eight weeks of the cholecystogram, 9 had uptakes under 15 per

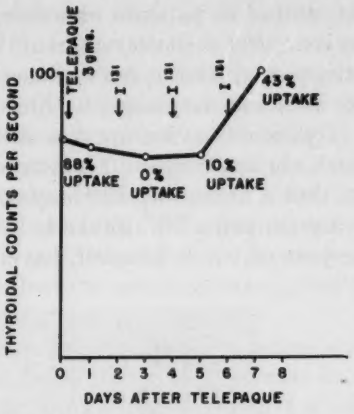

FIG. 7. The effect of iopanoic acid (Telepaque) upon the uptake of radioactive iodine in a patient with thyrotoxicosis. Counts per second are plotted on a semilog scale.

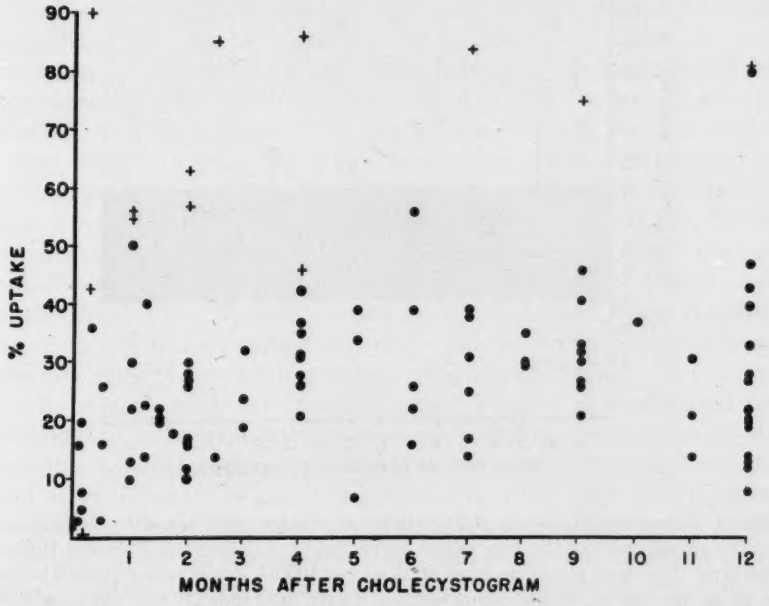

FIG. 8. The 24-hour uptakes of radioactive iodine in 102 patients at varying intervals after cholecystography. (The crosses indicate patients with hyperthyroidism.)

cent; and of 65 patients who were given radioactive iodine eight weeks to one year after a cholecystogram, 10 had uptakes under 15 per cent. Of 72 patients who were given radioactive iodine between four weeks and one year after a cholecystogram, three had uptakes of 10 per cent or under; and of 17 patients having uptakes determined up to and including four weeks after a cholecystogram, 7 had uptakes of 10 per cent or below. It is apparent, that a normal uptake may occur as early as three days after a cholecystogram and a low uptake as long as one year later. The low uptakes at one year may not, however, have been due to the cholecystography.

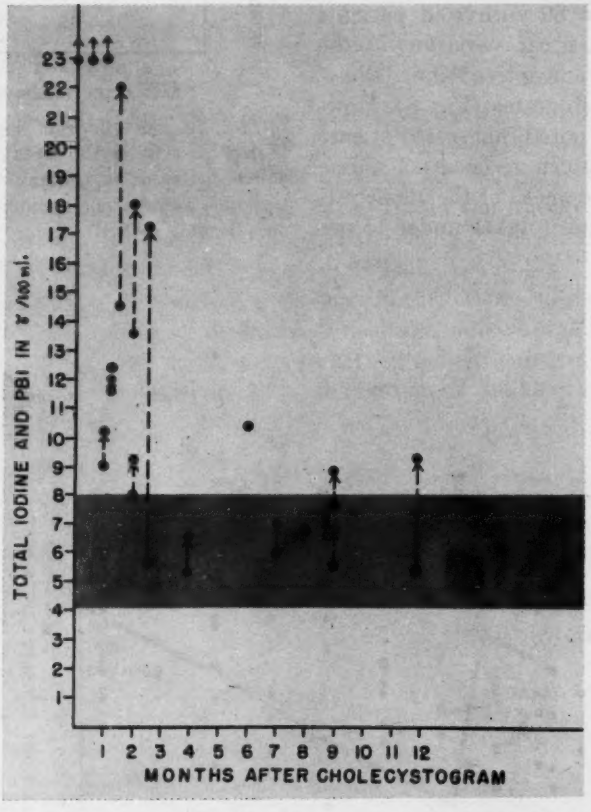

FIG. 9. The serum PBI levels of 21 patients at varying intervals after cholecystography. The upper point of the two values connected by a dotted line indicates the total iodine level. The three points recorded at 23 μg. per 100 ml. are an undetermined amount over 20 μg. per 100 ml. Single points without dotted lines indicate that the total iodine level was the same as that of the PBI. Normal values of PBI concentration are considered as 4–8 μg. per 100 ml.

Determinations of the serum PBI level were made on 21 euthyroid patients following cholecystograms (Fig. 9). In 9 patients in whom measurements were made at two months or less following cholecystography, all PBI levels were over 8 μg. per 100 ml. Of 12 patients whose PBI determinations were made between two months and twelve months following a cholecystogram, only 1 had a PBI concentration over 8 μg. per 100 ml. The largest measured discrepancy between PBI and total iodine levels occurred at ten weeks; but undoubtedly the high PBI values at one day, two weeks, and four weeks (over 20 μg. per 100 ml.) would have demonstrated an even more marked difference had this aspect of the matter been investigated at these times.

Of 13 hyperthyroid patients who were given radioactive iodine within one year of cholecystograms, 2 had uptakes below 46 per cent; in 1 of these it was 43 per cent at seven days, and in 1 it was zero at five days. At seven days 1 patient had an uptake of 91 per cent.

DISCUSSION

Although marked variation in the impairment of thyroidal iodine accumulation following cholecystography has been previously noted (6), there has been an impression of frequent long-standing reduction in the uptake of radioactive iodine. It was felt, therefore, to be of interest to study the half-life of the opaque material used in cholecystography, its mode of excretion, and its effect on iodide metabolism at varying intervals following ingestion. The half-life of radioactive Priodax in the rat, guinea pig, and human being is short—even briefer than that of a tracer dose of radioactive iodine. Deiodination seems to occur to a significant extent in the rat and guinea pig, and high levels of serum iodide may block the thyroid thus permitting more rapid excretion of iodide by the kidneys. The major urinary excretory product, however, is Priodax glucuronide. The shorter half-life of Priodax in the first day or so may be related to a rapid excretion of the Priodax not "bound" to protein. In the human being, deiodination seems to occur to a minimal extent and the short half-life seems based on the fast urinary excretion, mainly of Priodax glucuronide.

The relative difficulty in deiodinating Priodax is emphasized by the finding that an *in vitro* system of peroxidase and hydrogen peroxide rapidly deiodinates diiodotyrosine but slowly deiodinates thyroxine, Priodax, and and other iodinated organic compounds (7). Free, Page and Woollett (8) measured a 74 per cent excretion of intravenously administered pheniodol (Priodax) in the rat during three days, and found that 80–90 per cent was organic. Their studies indicated a higher percentage of excretion in the feces than in the urine. Intraduodenal Priodax in the cat was excreted to the extent of 45 per cent in six hours, more in the bile than in the urine.

Bang and Georg recovered 64–72 per cent of orally administered pheniodol in the feces of normal human beings (9). The present study indicates little deiodination in the human being and a fast excretion, mainly in the urine, of Priodax and Priodax glucuronide.

The reduction by Priodax of the uptake of radioiodine in the rat was similar to that produced by an equivalent amount of iodide. After the administration of 3 mg. of Telepaque, the uptake of radioactive iodine had not returned to normal in one week, but was normal or above normal in two weeks.

Although radioactive iodide was not detected in the serum of human subjects, it was found in the urine and presumably was present in the serum. There is evidence that most of the Priodax in human serum becomes associated with protein, or "bound" (1), but the foregoing determinations of serum total iodine and PBI concentration indicate a large amount of "unbound" material, a small amount of which is presumably iodide. The protein-binding capacity of serum for Priodax may be limited, and it would seem that at lower levels of PBI a larger proportion of Priodax is bound. Priodax can be distinguished from thyroxine by polarography (10), by electrophoresis (1), and by acetone extraction (11). *In vivo*, the hypophysis distinguishes Priodax from thyroxine, in that propylthiouracil-induced goiters still occur in the presence of large quantities of Priodax.

The evidence indicates a faster turnover of iodide in the hyperthyroid than the euthyroid patient and, therefore, an early return to normal or elevated levels of thyroidal radioactive iodine uptake following the ingestion of iodine compounds. After one week following a cholecystogram, hyperthyroid patients were noted to have uptakes in the high-normal or high range; after four weeks most euthyroid patients had uptakes in the low-normal or normal range. The wide variation between individuals (6) in the effect of Priodax on uptakes of radioactive iodine possibly may reflect different rates of Priodax glucuronide excretion, rates of deiodination, rates of iodide excretion and uptake, thyroxine formation, storage and metabolism, and amounts of iodide in the diet both before and after Priodax ingestion. The occasional long influence of inorganic iodide *per se* has been noted (12), as indicated by a depression of radioactive iodine uptake for six months in a patient following the ingestion of inorganic iodide sporadically over a period of years. Some cases of goiter formation and myxedema demonstrate a prolonged suppression of thyroid function following the ingestion of organic iodine compound. It has been assumed that this diminution in thyroid function is related to the inorganic iodide released from the organic compound, but why sporadic instances of such a suppression occur is not understood.

SUMMARY

Radioactive iodoalphionic acid (Priodax) given to animals and human beings was rapidly excreted. The half-life was five hours to four days in the animals, and twelve hours in the human being. The compound was partially deiodinated but was excreted mainly as Priodax and Priodax glucuronide. The period of depressed thyroidal uptake of radioactive iodine following administration of Priodax in the rat was usually one to two weeks, in the euthyroid human subject usually up to four weeks, and in the hyperthyroid patient one week or less.

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INFLUENCE OF IODOALPHIONIC ACID (PRIODAX), WITH AND WITHOUT THYROTROPIN, ON THYROIDAL I^{131} UPTAKE IN EUTHYROID PATIENTS*

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THE ingestion or administration of iodine-containing solutions is known to suppress thyroidal radioactive iodine uptake in euthyroid individuals. Werner *et al.* (1) state that a low uptake may persist up to twelve weeks after administration of stable iodine. Keating *et al.* (2) observed that the ingestion of Priodax (an organic iodine-containing compound used in cholecystography) resulted in low thyroidal radioiodine accumulation for periods ranging from a few weeks to many months. Drummy (3) reported reduction of the radioiodine uptake to less than 15 per cent for at least six months in a majority of 29 euthyroid patients after the ingestion of Priodax. Our experience in the Isotope Clinic at Letterman Army Hospital suggested that the inhibiting effect of Priodax is of shorter duration. Many patients who undergo thyroid function studies have had recent cholecystography with Priodax. Because of this, we felt it important to attempt a more accurate determination of the length of the period during which thyroidal I^{131} uptake is diminished after the ingestion of this compound.

Stanley and Astwood (4) showed that administration of thyrotropin (TSH) causes an increased rate of radioiodine uptake in euthyroid patients, even during therapy with large doses of thyroid. Jefferies *et al.* (5) reported that skin contact with iodine, or the ingestion of 0.3 cc. of a saturated solution of potassium iodide per day, reduced the thyroidal uptake of radioactive iodine both before and after TSH in euthyroid individuals. They felt that uptake values are invalid for six to eight weeks after the last exposure to iodine.

The administration of TSH has been useful in the differential diagnosis of primary and secondary hypothyroidism (5, 6, 7). It has been suggested as a means of determining the amount of potentially functioning thyroid

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tissue in patients receiving thyroid medication (4). Because, after administration of TSH, patients with primary hypothyroidism do not respond normally and euthyroid patients (even when taking large doses of thyroid) have normal thyroidal I^{131} uptakes, we considered using TSH to overcome the suppression of I^{131} uptakes caused by Priodax.

MATERIAL AND METHODS

Of the 11 subjects studied, all were euthyroid clinically, had taken no iodine-containing materials, and had an initial thyroidal I^{131} uptake within the normal range of 15 to 40 per cent. This initial uptake value served as the patient's base line (denoted as B, Figs. 1 and 2).

Six 0.5-gram tablets of iodoalphionic acid (Priodax) were ingested by each patient on day 0 of the test. At recorded intervals thereafter, the patients received tracer doses of carrier-free radioactive iodine (I^{131}) orally. Twenty-four hours later the concentration of radioiodine was determined with a scintillation counter held 15 cm. from the neck. Corrections were made for background and residual radioactivity from previous doses.

The 11 subjects were divided into two groups, A and B, by selecting them alternately. Group A (Fig. 1), consisting of 6 patients, was studied in an attempt to evaluate the degree and duration of the suppression of I^{131} uptake induced by ingestion of Priodax. At intervals of three to seven days during the first month, and seven to fourteen days during the second month, thyroidal I^{131} uptakes were determined. The study was

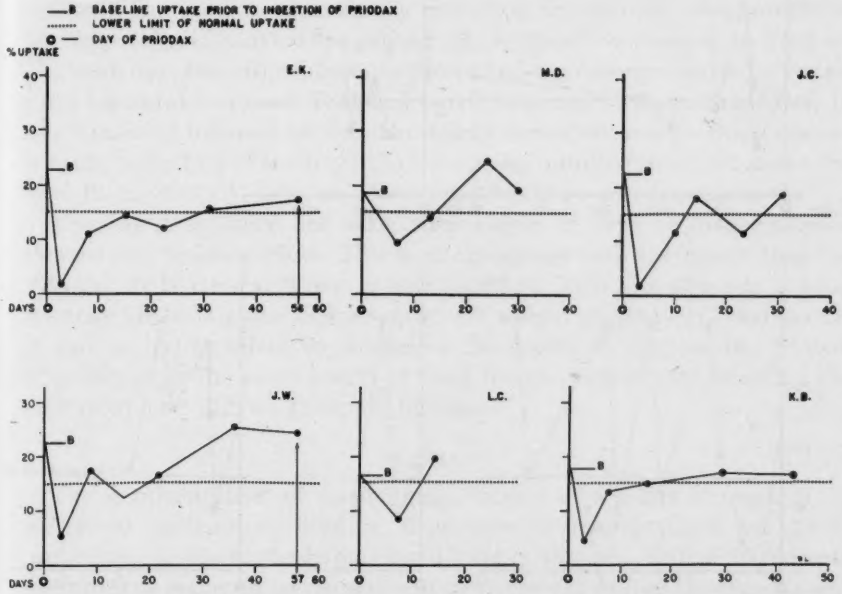

FIG. 1. Effect of ingestion of a single dose (3 Gm.) of Priodax on the 24-hour thyroidal uptake of I^{131} in 6 euthyroid patients.

terminated when a patient's low uptake rose to or above his base-line level before thirty days, or when it was within the euthyroid range after thirty days.

Group B (Fig. 2), consisting of 5 patients, differed only from group A in having determinations before and after administration of TSH. This group was studied in an attempt to discover whether or not administration of TSH would overcome the suppression induced by Priodax. At weekly intervals after the ingestion of Priodax, determinations of 24-hour thyroidal I^{131} uptakes were made in each patient. Each was then given 5 units of TSH intramuscularly. The next day another tracer dose of I^{131} and determination of 24-hour uptake was recorded (Fig. 2). One patient (T.M.) was followed for only eleven days. He is included only with reference to his response to TSH. The other 4 were followed until their uptakes without TSH rose above their base-line levels before thirty days, or were within the euthyroid level after thirty days. An attempt was made to keep the tracer doses of radioactive iodine as small as possible, consistent with accurate results. The doses varied from 11 to 58 microcuries; the majority were less than 30 microcuries. The number of tracer doses per patient varied from 3 to 11. Only 1 patient received more than a total of 200 microcuries of I^{131} during the period of study.

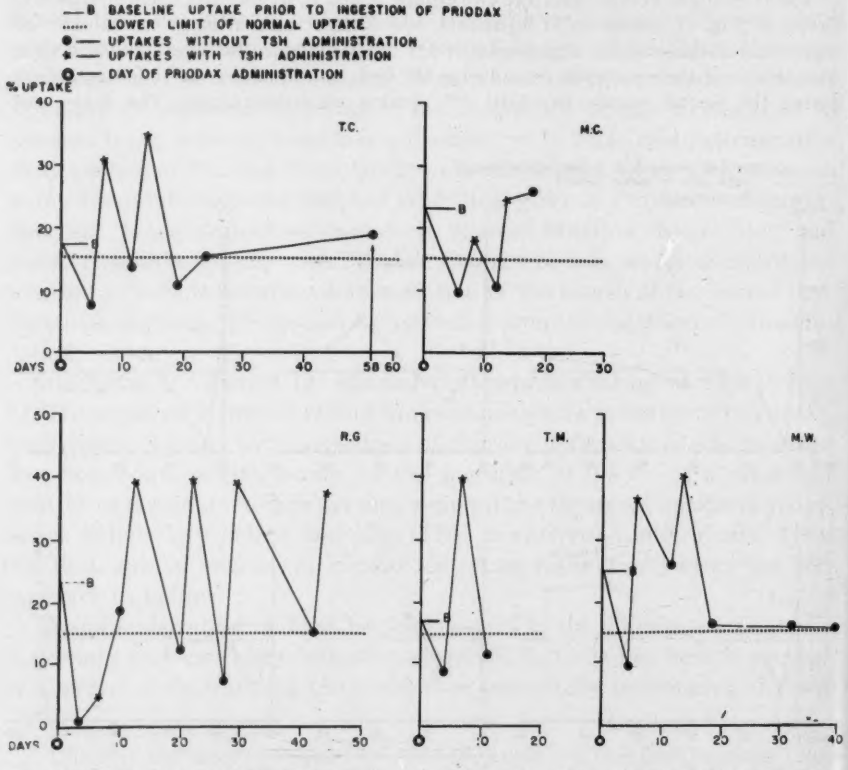

FIG. 2. Effect of thyrotropin (TSH) in overcoming the depression of the 24-hour thyroidal I^{131} uptake induced by Priodax in 5 euthyroid patients.

RESULTS

All 11 subjects had initial base-line 24-hour thyroidal I^{131} uptakes (denoted as B in Figs. 1 and 2) within the euthyroid range of 15 to 40 per cent, and were clinically euthyroid. The uptakes were reduced to less than 10 per cent in all patients during the first week after administration of Priodax.

Group A (Fig. 1)

Five of the 6 patients were followed for at least thirty days after ingestion of Priodax. In all, the thyroidal I^{131} uptake had returned to the euthyroid level within that period of time. Further studies at from thirty-six to fifty-seven days in 3 of these patients indicated that the uptakes remained normal. One patient (L.C.), who was followed for only fourteen days, had an uptake above his base-line value at the time that his study was discontinued.

Group B (Fig. 2)

Three of the 5 patients were followed for more than thirty days, 1 (M.C.) for twenty days, and 1 (T.M.) for eleven days. They received 11 doses of thyrotropin, following 10 of which the thyroidal uptakes rose to normal. The uptakes immediately preceding thyrotropin were usually in the hypothyroid range. One patient (R.G.) failed to respond to TSH on the sixth day, but after subsequent doses had a good response; at forty-two days his uptake without TSH had barely returned to the normal level. In the 2 patients followed for less than thirty days there was good response to TSH in both. In 1 of them (M.C.) the uptake returned to a level above the base line at twenty days, and therefore the study was discontinued.

Uptakes determined one week after a dose of TSH no longer demonstrated any residual effect. This is in agreement with the report that the effect of TSH lasts for three to four days (4). TSH was effective in temporarily abolishing the depression of I^{131} uptake caused by Priodax, but it had no lasting effect in shortening the period of suppression. It took approximately the same length of time for the uptakes to return to the euthyroid level in both groups of patients.

COMMENT

The administration of six 0.5-gram tablets of Priodax to each of 11 euthyroid patients resulted in depression of the thyroidal uptake of radioactive iodine to the hypothyroid level in all cases. In 9 of 10 patients the uptakes returned to normal within thirty days after the ingestion of Priodax, indicating that uptakes determined one month after administration of this substance for cholecystography should be reliable. Within the

first month after ingestion of Priodax, the administration of TSH was followed by normal uptakes in 9 of 10 instances. In the 1 patient (R.G.) in whom there was no response to TSH at six days, the response was normal at eleven days, and following each dose thereafter. R.G. was the only subject in whom the uptake did not return to a normal level within thirty days after the ingestion of Priodax.

SUMMARY AND CONCLUSIONS

The findings in a series of 11 patients indicate that iodoalphionic acid (Priodax) suppresses the thyroidal uptake of radioactive iodine for less than thirty days in euthyroid subjects. Thus, thyroidal uptakes determined beyond thirty days after the use of Priodax for cholecystography should be reliable in most cases.

In thyroid function tests undertaken within the first month after cholecystography, the administration of thyrotropin is of value in temporarily overcoming the suppressive effect of Priodax. Eventually, the I^{131} uptake rises spontaneously to the euthyroid level, regardless of whether or not thyrotropin has been administered.

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ENDEMIC GOITER IN VENEZUELA STUDIED WITH I^{131}*

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ENDEMIC goiter has been thoroughly investigated with the aid of radioiodine by American and Argentinian workers (Stanbury *et al.*, 1, 2). It has been shown by them that, in patients with goiter from the region of Mendoza in Argentina, the thyroid gland exhibits an increased avidity for iodine, with a rapid turnover which can be further accelerated by administration of thyrotropin. These findings are consistent with the theory which implicates iodine deficiency as the chief factor in the genesis of endemic goiter and eliminates, at least in the region studied, the possibility of a goitrogenic factor which would block the trapping of iodine by the thyroid gland.

We were interested in confirming the finding of increased iodine avidity by studies in another endemic goiter area, and in ascertaining whether or not there existed a difference in thyroidal radioiodine uptake between goitrous and nongoitrous subjects from the same region. Although, in previous studies, no defect in the "iodine trapping mechanism" of the thyroid had been shown to exist, it remained to be seen whether the organic binding of the trapped iodine was unimpaired. Finally, the magnitude of I^{131} incorporation into plasma protein had to be studied, since Stanbury and his collaborators were unable to do so because they lacked sufficiently sensitive apparatus at the time of their study. In the present investigation we were able to explore this last point only in a preliminary way.

In addition to studies with radioiodine, the expedition from this laboratory carried out statistical (3), clinical and laboratory (4), dietetic (5) and psychologic (6) studies, which are reported elsewhere. Some of the results described have been published in abstract form (7).

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* Part of this study was presented at the Annual Meeting of The Endocrine Society, Atlantic City, New Jersey, June 2–4, 1955.

SITE OF THE INVESTIGATION, DESCRIPTION OF PATIENTS, AND METHODS USED

Venezuela, situated in the northern region of South America (Fig. 1), receives an important lateral range of mountains from the Andes, covering much of the country's western region where several peaks reach 15,000 feet. The cross-hatched area on the map indicates the region where goiter has been known to exist for at least two centuries and where accurate statistics in schoolchildren are now available (3). Within this region, to the west of Mérida, the town of Bailadores was chosen for the present study.

FIG. 1. Map of Venezuela. The cross-hatched area indicates the region where endemic goiter has been shown to exist.

Bailadores is the center of a fairly large rural area, and lies in a narrow and fertile valley running north-south and rising from approximately 3,000 to 6,000 feet. The town itself lies at 4,800 feet. The number of inhabitants in the valley of Bailadores is approximately 10,000, most of them small farmers, of white or mestizo blood. From the following observations it is clear that Bailadores is an area of high endemicity:

In a group of 1359 subjects whose thyroid was examined, there were 718 adults (398 men and 320 women) and 641 schoolchildren under 17 years of age (312 boys and 329 girls). Goiter was present in 83 per cent of the total group—in 81.2 per cent of the men, 88.8 per cent of the women, 77.2 per cent of the boys, and 85.3 per cent of the girls.

Radioiodine studies. Of the 86 goitrous adults among the 110 studied

with I^{131}, only 10 had very large, obvious goiters; 20 had visible goiters; and 56 had palpable goiters. The remaining 24 adults had no goiter. In none of the 28 children was the thyroid more than barely visible.

In 46 goitrous subjects in whom a satisfactory basal metabolic rate (BMR) could be obtained, the average value was −14.2 per cent, with a large standard deviation of 16.8 per cent. In 11 nongoitrous subjects, the average BMR was −15.5 per cent, with a standard deviation of 15.2 per cent (4).

Nutrition was generally inadequate. The average caloric intake was 1899 calories per day—protein 66 Gm. (63.7 per cent of vegetable, and 36.3 per cent of animal origin), carbohydrate 333 Gm., and fat 15 Gm. per day. Calculated daily vitamin A intake was 3872 I.U. per day. No known goitrogen was detected in the diet (5).

In every case, the tracer dose of radioiodine was administered orally. The iodine was standardized and furnished by the Abbott Laboratories, Oak Ridge. In adults, a dose of 50 microcuries was used, except for the plasma studies, in which a dose of 100 microcuries was employed. In children and in members of the expedition, the dose was 25 microcuries. Thyroid activity was measured at appropriate intervals with a scintillation detector (Nuclear, Chicago, Model DS–1) placed 40 cm. away from the thyroid notch. This value was "corrected" by multiplying it by the factor 0.85 (2), and then subtracting from the resulting value the "background" obtained 40 cm. away from the thigh. Results were expressed as a percentage of the administered dose, using a 50-cc. aliquot of the same solution, measured in a 250-cc. beaker at the same distance and location, and corrected for background. Urinary radioactivity was read with a shielded Nuclear DS–1 scintillation counter, and the result compared with an appropriately diluted standard. Plasma proteins were precipitated with 10 per cent trichloroacetic acid, washed twice with the same substance and the precipitate redissolved in ammonium hydroxide to a volume of 10 ml. Radioactivity was counted in an aluminum cup with a shielded DS–1 Nuclear scintillation detector. For counting the thyroid nodules, a lead collimator with a 1-inch opening was placed on the detector and applied on the center of the nodule, and later on the non-nodular thyroid tissue for comparison. Only nodules which were clinically well defined as to size and contour were chosen for the study.

For the study of the effect of potassium perchlorate on thyroidal activity, 27 subjects from Bailadores and 6 control subjects from the expedition were given 50 microcuries of I^{131}, after which thyroid counts were performed at two, four, five, six and seven hours. Seven subjects from Bailadores received no drug except radioiodine, serving as controls. To other subjects, we gave 100 mg. of potassium perchlorate, to 7 others 300 mg., and to 6 others 500 mg. after the four-hour count. Five members of the expedition were similarly studied and received a dose of 300 mg.

RESULTS OF I^{131} STUDIES

On the right side of Figure 2 are shown the distribution curves of the 48-hour thyroidal radioiodine uptake in 106 adults from Bailadores, with

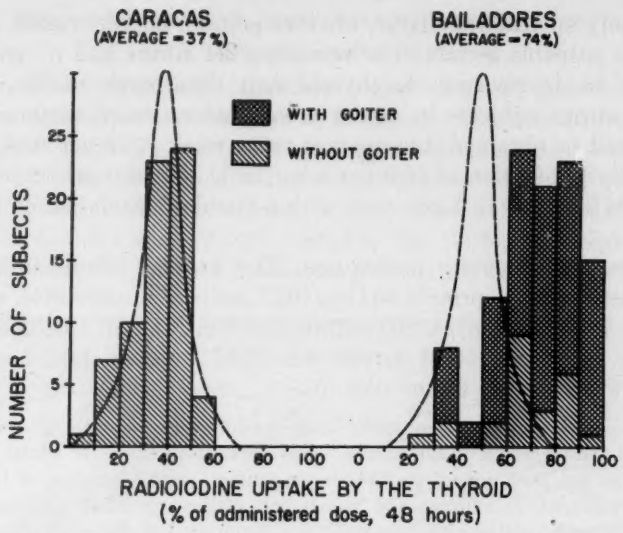

FIG. 2. Histogram of the frequency distribution of thyroidal 48-hour radioiodine uptake in 70 euthyroid adults of Caracas, the capital (left), and 106 goitrous and nongoitrous adults of Bailadores in the Andes (right). The bell-shaped curves represent the frequency distribution in Boston, Massachusetts as given by Skanse (8).

and without goiter. The bell shaped broken-line curves represent the distributions of this same value in Boston, Massachusetts, as reported by Skanse (8). On the left side of the figure, superimposed upon the Boston curve, are the values obtained by us in Caracas, the capital of Venezuela, in 70 adult euthyroid subjects (9).

In Figure 3 is shown the distribution of thyroidal uptakes among 28 Bailadores schoolchildren.

In Figure 4 is shown the distribution of the percentages of the administered dose of I^{131} excreted in the urine of adult subjects in whom we were able to obtain satisfactory collections.

TABLE 1. AVERAGE AND STANDARD DEVIATION OF THYROIDAL UPTAKE AND URINARY EXCRETION OF RADIOIODINE IN GOITROUS *vs.* NONGOITROUS ADULTS OF BAILADORES

(per cent of administered dose in 48 hours)

Group	No. of subjects	Thyroidal uptake	No. of subjects	Urinary excretion
Without goiter	24	68±21	23	21±16
With goiter	86	76±10	77	17±13
Total	110	74±14	100	18±14

In Table 1, the 48-hour thyroidal I^{131} uptakes and urinary excretions of goitrous and nongoitrous adult subjects are compared.

Figure 5 illustrates the changes in thyroidal radioactivity effected by doses of potassium perchlorate ranging from 100 mg. to 500 mg.

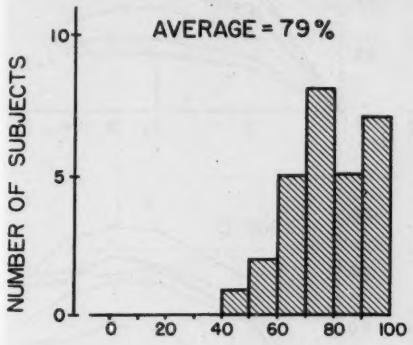

FIG. 3. Histogram of the frequency distribution of thyroidal 48-hour radioiodine uptake in 28 children of Bailadores.

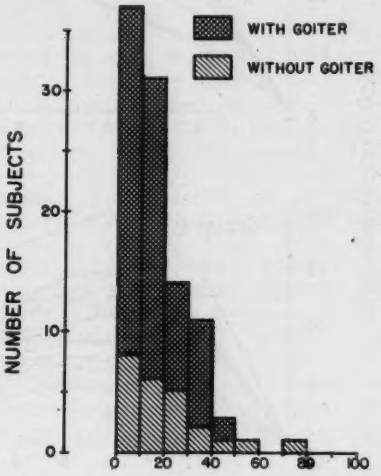

FIG. 4. Histogram of the frequency distribution of 48-hour urinary excretion of radioiodine (percentage of the administered dose).

Table 2 lists the counts obtained when the detector was applied directly over nodular, as contrasted with non-nodular areas in goitrous subjects.

In 8 subjects who were given 100 millicuries of I^{131} by mouth, the average level of plasma protein-bound iodine, twenty-four hours after administration, was 0.486 per cent of the administered dose per liter of plasma, with a range of 0.134 to 1.007.

COMMENTS

The 48-hour thyroidal I^{131} uptake

It may be seen from Figure 2 that in the 70 euthyroid subjects studied in Caracas (with the same methods and apparatus as used in Bailadores) the distribution of thyroidal I^{131} uptake was similar to that obtained by Skanse in Boston, whereas the Bailadores values were markedly higher—the majority lying above the 60 per cent level. Our subjects had, on the whole, higher uptakes than those reported by Stanbury *et al.* for Mendoza in Argentina (1, 2). Thus, in Mendoza, of 118 subjects studied, only 11 had a

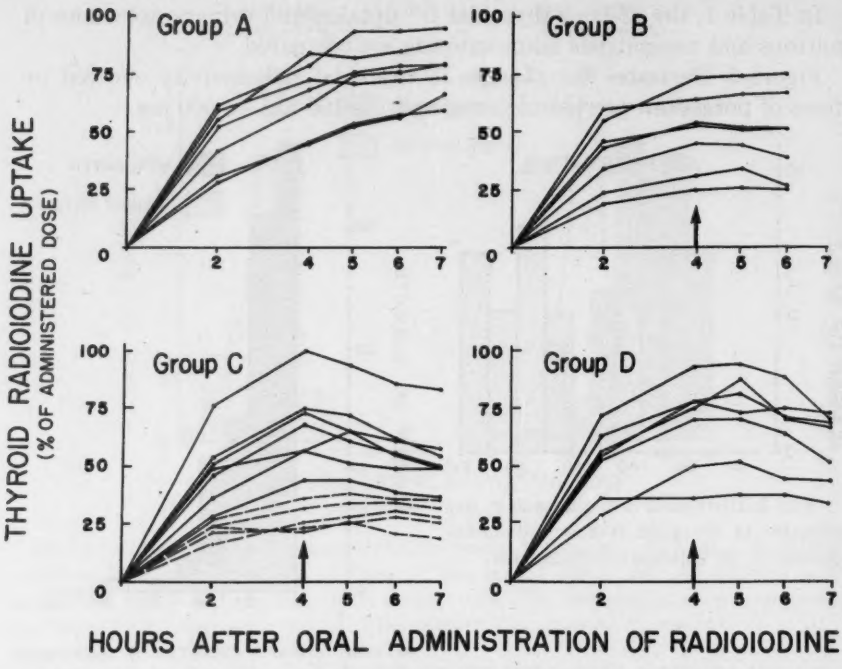

FIG. 5. Effect of potassium perchlorate given by mouth on thyroidal activity. Group A received no perchlorate; Group B, 100 mg.; Group C, 300 mg.; and Group D, 500 mg. The time of administration of perchlorate is indicated by the arrow. The interrupted lines in Group C represent thyroidal uptakes in members of the expedition from Caracas.

48-hour uptake higher than 80 per cent, and none had an uptake higher than 90 per cent. In our material, of 110 subjects studied, 38 had a 48-hour uptake higher than 80 per cent, and in 15 of these the uptake was over 90 per cent. It may be that iodine deficiency is more profound in Bailadores than in Mendoza. On the other hand, Mendoza is a fairly large city, in which the goiter problem has been studied for a long time, and it is possible that the use of iodinated compounds is more widespread than in Bailadores.

The 48-hour I^{131} uptake was also determined in 10 members of our expedition during their stay in Bailadores. In 9 of them the uptake ranged from 33 to 46 per cent of the ingested dose; in 1 it was 64 per cent. Thus, with 1 exception, all had a normal uptake. The 1 member with a high uptake was a laboratory helper who came from the Universidad de los Andes in Mérida, a region where goiter is also endemic, although to a lesser degree than in Bailadores. The other 2 members from Mérida did not have an abnormally high uptake.

In 4 subjects whose thyroid activity was counted every few hours,

TABLE 2. RADIOACTIVITY COUNTED DIRECTLY OVER THE SKIN IN THE REGION OF THE THYROID (COUNTS/MINUTE)

Patient	Sex	Dose of perchlorate at hour 4 after I^{131}	Region of thyroid*	Hours after ingestion of 50 μc. I^{131}				
				2	4	5	6	7
V.A.	M	0	N	9,610	11,308	10,431	10,799	10,774
			T	3,610	5,457	6,628	10,374	8,894
F.M.Y.	F	0	N	3,816	7,991	9,527	10,572	10,904
			T	5,531	11,370	12,901	10,002	14,658
M.S.	F	0	N	5,538	7,206	11,553	10,196	10,743
			T	9,771	8,889	11,652	10,310	8,322
P.S.	M	100	N	4,740	5,051	5,509	6,349	—
			T	5,334	8,668	10,612	10,100	—
A.R.	F	100	N	10,413	14,996	21,167	23,476	—
			T	18,182	19,381	8,130	12,422	—
E.V.	F	100	N	12,628	31,185	26,367	29,143	—
			T	20,322	22,037	27,374	15,706	—
R.S.	M	100	N	1,358	1,653	2,352	4,008	—
			T	4,642	4,507	5,907	10,245	—
A.R.	M	300	N	1,938	2,573	2,447	2,398	2,265
			T	11,904	16,645	18,306	13,208	16,219
R.R.	M	300	N	3,149	5,445	6,400	5,033	6,747
			T	7,129	12,000	10,847	9,743	8,146
H.M.	F	300	N	17,925	26,546	24,698	17,128	20,800
			T	17,115	16,803	14,936	17,244	16,511
E.R.	M	300	N	784	876	975	997	957
			T	971	1,142	1,278	1,180	1,583
O.M.	F	500	N	11,234	13,063	16,534	14,713	11,625
			T	15,648	23,585	16,382	13,107	13,255
M.C.	F	500	N	12,082	18,647	15,235	14,747	14,665
			T	9,446	14,396	13,440	13,653	8,101
A.V.	M	500	N	17,448	27,973	28,968	25,473	21,529
			T	17,945	25,498	24,247	18,044	22,562
M.E.	F	500	N	—	14,576	12,232	9,438	3,908
			T	—	18,107	22,700	25,075	25,892

* N: Node.
T: Non-nodular tissue.

there was a brisk uptake during the first few hours, so that by the fourth hour most of them had already accumulated over 60 per cent of the dose administered. There was a similar finding in the subjects studied with perchlorate (Fig. 5). After the first ten or twelve hours, there was a gradual slow decrease, so that activity at forty-eight hours was generally somewhat below that on the first day.

In the 28 children studied (age 8 to 14) the thyroidal uptake was also definitely elevated (Fig. 3).

Urinary excretion of I^{131}

As might be expected, the urinary values were low, since a large proportion of the dose had been removed by the thyroid. It is likely that our values are lower than they should be, as we could never be certain of the completeness of collections. In general, however, the subjects were cooperative.

Goitrous versus nongoitrous subjects

Among adult subjects studied, 24 had no goiter; indeed, they were chosen from a group of people in whom, although they had always lived in Bailadores, the thyroid could not be palpated. In Figures 2 and 4, it may be seen from the respective distributions of the singly and doubly cross-hatched areas that there is no significant difference between goitrous and nongoitrous subjects with respect to thyroidal radioiodine uptake and urinary excretions. A large proportion of nongoitrous subjects had uptakes well over 60 per cent. A typical case is that of A. G. (Fig. 6), a 59-year-old man, apparently euthyroid, with a thyroidal uptake of 88 per cent at forty-eight hours. In children, the thyroidal 48-hour activity was somewhat lower in the nongoitrous compared with the goitrous, but the difference was not statistically significant.

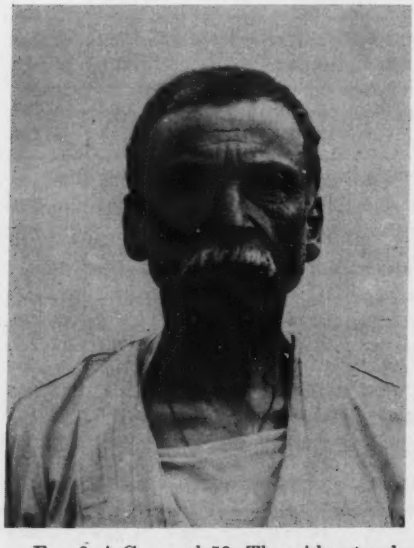

FIG. 6. A.G., aged 59. Thyroid not palpable. The 48-hour thyroidal uptake of radioiodine was 88 per cent of the administered dose.

Thus, most of the subjects in the endemic area studied, whether they had palpable goiters or not, presented a markedly increased thyroidal avidity for iodine. That this avidity was the result of iodine deficiency is

probable, although other contributing factors may have been operating also. We did not determine the iodine content of water and soil, or the urinary stable iodine excretion. De Venanzi and his co-workers (10) have previously shown in three samples from different drinking sources in a similar region of the Venezuelan Andes, values of 4.22, 1.50 and 1.36 micrograms of iodine per liter. In their clinical material, 13 nongoitrous subjects had an average excretion of 17.78 ± 1.86 (standard error) micrograms per liter, versus 11.63 ± 1.23 in 20 patients with diffuse goiter and 11.09 ± 2.76 in 20 patients with nodular goiter. All these values are definitely low. In a more recent study carried out by ourselves in Bailadores (11) the average urinary 24-hour I^{127} excretion was found to be 22.5 micrograms per day, with a range of 9.0 to 40.0 micrograms, in 14 subjects.

Why goiter develops in some individuals and not in others has not been made clear by the present study. There was no major difference between the two groups with respect to history, physical examination, basal metabolic rate and other laboratory studies (4). The number of subjects who had left Bailadores at one time or another for periods of more than fifteen days was rather more numerous in the nongoitrous group, but the number in this group was too small to make this difference convincing. It is likely that most of the subjects studied suffered from relative iodine deficiency, so that mechanisms developed in the thyroid gland which enabled it to trap efficiently the small amounts of iodine offered by diet and water; and that in most of them this process was accompanied by an increase in weight and size of the gland. A few of the thyroids were evidently able to carry out this efficient iodine extraction without detectable anatomic hypertrophy.

Effect of potassium perchlorate on thyroidal I^{131} uptake

That the iodine-trapping mechanisms are unimpaired in endemic goiter is clear from the previous account. During the first few hours after radioiodine administration, the curve of uptake by the thyroid in 7 subjects showed a constant upward trend (Fig. 5, Group A); when a dose of 100 mg. of potassium perchlorate was given at the fourth hour (Fig. 5, B) the uptake curve flattened to a plateau, indicating that no more iodine was being trapped by the thyroid, or that the amount of iodine trapped was approximately equivalent to the amount released by the thyroid. With doses of 300 and 500 mg. of perchlorate (Fig. 5, Groups C and D), the thyroidal radioactivity curves showed a definite downward trend, so that in some cases as much as 26 per cent of the dose had been discharged three hours after administration of the drug. In 5 members of the expedition (Group C, dotted lines) this phenomenon was not observed with 300 mg. of perchlorate. Broadly speaking, the higher the uptake, the greater the decrease after perchlorate.

There are at least two possible explanations for these observations. It is known that thiocyanate (12) or perchlorate (13) blocks the trapping mechanism so that, if administered to a subject with a block at the organic-binding level, there is a subsequent emptying of the thyroid of all of the unbound iodine. If radioiodine has previously been picked up by the thyroid, this is reflected by decreased thyroidal activity. If we assume that in endemic goiter release of iodine from the thyroid in the form of thyroid hormone is greatly accelerated because of increased turnover, one would expect a decrease in thyroidal activity when the supply of inorganic radioactive iodine is suppressed or curtailed through the administration of such a drug as perchlorate. On the other hand, the decrease in thyroidal activity after perchlorate may indicate that some of the iodine accumulated in the thyroid has not been bound to protein and is thus "washed out" after the trapping mechanism has been blocked. This would indicate a block at the level of the "binding mechanism," although this block would not be total; a large portion of the iodine finds its way (probably rapidly) into the organic moiety. This is evident from the clinically euthyroid, or at most slightly hypothyroid state of the majority of the subjects studied, and from our preliminary plasma I^{131} studies.

Two endemic cretins were studied in a similar way with perchlorate. In both, thyroidal I^{131} uptake reached a maximum at five hours, being 35 per cent in one (nongoitrous) and 57 per cent in the other (goitrous). Perchlorate failed to reduce activity in either. Thus these cretins, unlike the sporadic goitrous cretins reported by Stanbury and Hedges (14), had no demonstrable defect in organic binding mechanisms.

Level of plasma protein-bound I^{131} at twenty-four hours

The level of plasma protein-bound I^{131} in our 8 subjects was well within the "hyperthyroid range" reported previously in the literature. Thus, the normal 24-hour PBI^{131} level is stated to be 0.022 per cent (range, 0.005 to 0.055) of the dose per liter of plasma by Schultz *et al.* (15) and 0.091 per cent (range, 0.076 to 0.151) by Silver *et al.* (16). For hyperthyroid patients, Schultz *et al.* reported a value of 0.351 per cent (range, 0.059 to 1.440): and Silver *et al.*, 1.087 per cent (range, 0.197 to 5.519).

We hesitate to draw definite conclusions, since our counting equipment was relatively inefficient. However, it appears that, in endemic goiter, the small amount of iodine presented to the thyroid is bound to protein more rapidly and in proportionately larger percentage than in subjects from nonendemic areas. More recent studies have confirmed this point (11). That this is true is further suggested by the fact that in Caracas, with the same methods and counting equipment, we were unable to obtain counts higher than background counts in the plasma of 7 euthyroid subjects.

Thyroid nodules

In Bailadores, 54 per cent of the goiters in adults were found to be nodular (3). Of 323 adult males with goiter, 53.6 per cent had palpable nodules; and of 284 adult females, 55.6 per cent had such nodules.

Fifteen nodular thyroids were studied, 12 of the subjects being given from 100 mg. to 500 mg. of perchlorate at the fourth hour after administration of radioiodine (Table 5). Among the 15 nodes studied, 12 yielded a count distinctly below that for non-nodular tissue. Thus they could be classified as "cold" nodules (17). When the subjects were given perchlorate, the results varied from increases to decreases, and in many instances there was no significant change. It is thus impossible to draw conclusions from the data. In none of the subjects not given perchlorate was there any lowering of the activity after the fourth hour.

SUMMARY

In the endemic goiter area of Bailadores in the Venezuelan Andes, 86 adults with goiter had an average 48-hour thyroidal radioiodine uptake of 76 per cent of the dose administered; the uptake was 68 per cent in 24 nongoitrous adults, and 79 per cent in 28 children. The difference between the uptakes in goitrous and nongoitrous subjects was not significant, either in the adults or the children. Urinary 48-hour radioiodine excretion in 23 nongoitrous adults was 21 per cent and in 77 goitrous adults 17 per cent.

When given at the fourth hour after the administration of radioiodine, potassium perchlorate in a dose of 300–500 mg. produced a definite decrease in thyroidal radioactivity in 11 of 12 cases studied.

The level of plasma protein-bound I^{131} at twenty-four hours in 8 subjects was within the range found by other authors in patients with hyperthyroidism.

In 12 of the 15 nodules studied by direct counting over the skin the radioactivity was less than in the non-nodular tissue. They could thus be classified as "cold" nodules.

It is concluded that, in this endemic area, most thyroid glands exhibit great avidity for iodine. This is interpreted as indicating the presence of iodine deficiency, although other factors may also be acting in the production of goiter.

Acknowledgment

We are greatly indebted to the Universidad de los Andes and to its Rector, Dr. Joaquin Marmol Luzardo, for their financial support and actual help in carrying out this study.

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HYDROCORTISONE METABOLISM IN THE PERFUSED ISOLATED RAT LIVER*

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IT HAS been abundantly demonstrated that the liver rapidly metabolizes hydrocortisone (compound F) both *in vivo* and *in vitro* (1–8). Alterations are effected both in the side chain at C_{17} and in saturation of double bonds in ring A.

Bile has been demonstrated to be an excretory pathway for metabolites of hydrocortisone, but the nature of these substances in bile has not been clearly characterized (8, 9). The work reported here represents data gathered by a study of this problem in the isolated perfused rat liver.

MATERIALS AND METHODS

A perfusion apparatus for the isolated rat liver was employed which has been described in detail in previous publications (10, 11). Hydrocortisone was determined in plasma by the Silber-Porter method (12). Hydrocortisone was determined in bile by the same chloroform extraction as used in dealing with plasma. When H_2SO_4 was added however, a cherry-red interfering substance developed in both the blank (extract plus H_2SO_4) and the sample (extract plus phenylhydrazine plus H_2SO_4). This substance was regularly found in bile from the perfusion apparatus but not in bile from bile-fistula animals. It was presumably due to products of the hemolysis which occurs to some degree during the perfusion. This interfering material could be eliminated almost completely by interposing the following steps. The chloroform extract of bile was washed with 1 ml. of 15 N H_2SO_4 in order to develop the colored substance, and then partially neutralized with 7 ml. of 10 per cent Na_2CO_3. After centrifugation the aqueous layer, which contained the major part of the colored substance, was discarded. The residual chloroform layer was washed with alkali and then processed in the usual fashion, as with plasma extracts. The absorption curve of such bile samples had a sharp peak at 410 mμ.

The addition of hydrocortisone (compound F) directly to rat bile in the test tube allowed complete recovery of the added steroid when the procedure followed was identical with that performed with perfusate samples. Analysis of bile from bile-fistula animals revealed no detectable free compound F.

RESULTS

Upon circulation of 166 micrograms (μg.) of compound F (Cortef, Upjohn—hydrocortisone in alcoholic solution) through the perfusion appara-

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tus, in the absence of the liver, the steroid levels did not change appreciably over a four-hour period, averaging 99 μg. per 100 ml. at four hours (Fig. 1).

Perfusion of the isolated hind limbs of a normal rat after addition of 166 μg. of compound F resulted in no appreciable change in steroid levels over a one-hour period. The steroid level at ten minutes was 57.4 μg. per 100 ml. and at one hour, 60 μg. per 100 ml. These are comparable to the levels found when blood is circulated through the perfusion apparatus in the absence of the liver, *i.e.*, when red blood cells are the only "tissue"

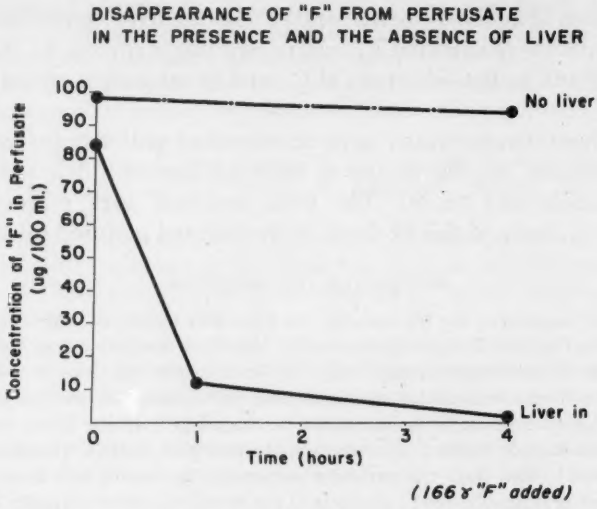

FIGURE 1

present. Hence, the perfusate levels were relatively stable in the presence of viable tissues other than the liver.

When a liver was perfused, and 166 μg. of compound F added to the perfusate, the steroid levels dropped promptly, averaging 84 μg. per 100 ml. initially, 12 μg. per 100 ml. in one hour, and 2 μg. per 100 ml. in four hours (Fig. 1).

When the amount of compound F added to the perfusion apparatus was 20 mg., the average steroid level in ten minutes was 6.3 mg. per 100 ml., and in one hour 0.53 mg. per 100 ml. (Fig. 2). This represents a disappearance rate of 91.4 per cent in fifty minutes.

The bile collected during such one-hour perfusions demonstrated steroid levels averaging 16.8 μg. per ml. or 1.68 mg. per 100 ml. (Table 1).

When the perfusion was performed in an atmosphere of 95 per cent nitrogen and 5 per cent CO_2 instead of 95 per cent oxygen and 5 per cent CO_2, the rate of alteration of the α-ketol side chain, although retarded,

TABLE 1. BILIARY EXCRETION OF HYDROCORTISONE (COMPOUND F) BY PERFUSED LIVER*†

Exper. number	Total bile volume (ml.)	"F" concentration in bile (μg./ml.)	Total "F" in bile
L.P. 242	1.60	16	25.6
L.P. 248	1.53	23	35.2
L.P. 249	1.84	14.8	27.2
L.P. 251	2.60	15.7	40.8
L.P. 252	1.29	14.7	19.0
Average:	*1.77*	*16.8*	*29.5*

* Duration of perfusion—one hour.

† Cortef (Upjohn), 20 mg., was added to each perfusate.

still proceeded at a considerable rate. After the addition of 166 μg. of compound F, steroid levels at the end of one minute were 60 μg. per 100 ml. in oxygen and 77 μg. per 100 ml. in nitrogen; at the end of ten minutes, 15 μg. per 100 ml. in oxygen and 23 μg. per 100 ml. in nitrogen; at one hour, 0 in oxygen and 23 μg. per 100 ml. in nitrogen.

When the bile duct of the perfused liver was ligated just before the prep aration was inserted into the perfusion apparatus, the rate of alteration of 20 mg. of added compound F was markedly slowed. At ten minutes the level was 8.1 mg. per 100 ml., and at one hour 4.75 mg. per 100 ml. (Fig. 2). This represents a disappearance rate of 40.5 per cent in fifty minutes.

When the bile-duct cannula was placed so that bile formed by the perfused liver was allowed to return directly to the perfusate, the rate of alteration of 20 mg. of added compound F was likewise markedly retarded, and was comparable to that seen in the preparation with the bile duct ligated. At ten minutes the steroid level was 8.2 mg. per 100 ml., and at one hour 5.5 mg. per 100 ml. (Fig. 2). This represents a disappearance rate of 33 per cent in fifty minutes.

DISCUSSION

The rate of metabolism of hydrocortisone (compound F), as measured by the Silber-Porter method, has been studied in the isolated perfused liver preparation. It is emphasized that this method measures the integrity of the α-ketol side chain and furnishes no information about metabolism of ring A.

In the presence of blood, but in the absence of liver, hydrocortisone is not appreciably altered over a four-hour period. In the presence of other tissue such as muscle, connective tissue, bone and skin, as in the perfusion

of isolated hind limbs, the level of added hydrocortisone is relatively stable over a four-hour period.

When the liver is perfused, however, hydrocortisone disappears rapidly in the first hour, and is present in only very small quantities at four hours. This demonstrates that the liver rapidly metabolizes hydrocortisone.

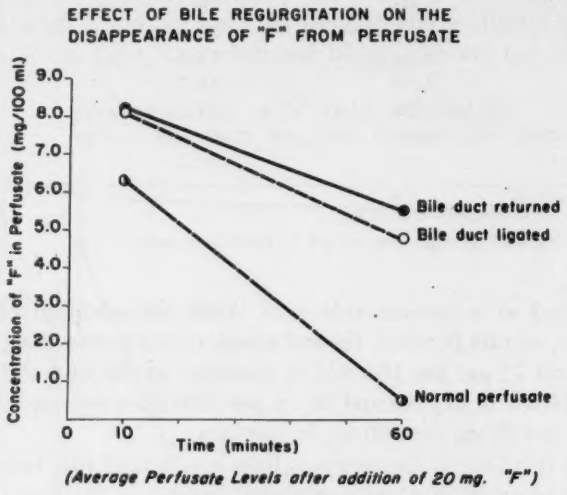

FIGURE 2

Perfusion under an atmosphere of nitrogen retards, but does not stop, this process.

When the function of bile removal is blocked, as by bile-duct ligation, or by return of bile into the perfusate (thus simulating the regurgitation type of jaundice), the rate of disappearance of hydrocortisone from the perfusate is markedly retarded.

Hydrocortisone is consistently detected only in extremely small amounts in bile secreted by the perfused isolated rat liver, even when high perfusate levels of hydrocortisone are used.

It would appear that free steroid appears in the bile, although in very much smaller amounts than that which disappears from the perfusate. When bile loss was prevented, or bile was returned to the perfusate, the levels of steroid in the perfusate were much higher, by an amount which could not be accounted for by the small quantities measured in the bile. This suggests that hydrocortisone is secreted in bile 1) as free steroid, 2) as a conjugated form of the α-ketol side chain which may be readily dissociated in the presence of blood, and 3) as degradation products which

do not react with the Silber-Porter reagents, but which can be demonstrated by radioactive tracer techniques (8).

SUMMARY

In the isolated perfused liver preparation, using the Silber-Porter method to measure hydrocortisone, it is demonstrated that: 1) the liver metabolizes hydrocortisone rapidly, whereas other tissues such as red blood cells and muscle do so at a much slower rate, 2) the liver secretes free hydrocortisone into the bile, and 3) regurgitation of bile into the perfusate markedly retards the rate of disappearance of hydrocortisone from the perfusate.

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DETERMINATION OF URINARY 11-OXYGENATED 17-KETOGENIC STEROIDS*

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THE increasing clinical investigative interest in adrenocortical function and awareness of therapeutic aspects of adrenal dysfunction have increased the need for specific methods of urinary corticoid assay which are adaptable to use in the hospital laboratory.

Certain problems are involved in applying any of the published methods (1–7) to clinical investigations. In attempting to choose the most appropriate method for our institution, three important functions were deemed desirable: 1) the method should be conveniently applicable to a sufficiently large number of specimens; 2) it should detect the greatest possible number of the known adrenocortical steroid metabolites of the C_{19} and C_{21} series with only a minimum number of compounds to be analyzed per specimen; and 3) the method should have high chemical specificity. It is the purpose of this communication to describe a new method of analyzing urinary corticoids, which embodies the advantage of measuring in one step a pair of isomeric oxidation products derived from the excreted 11-oxygenated cortical steroids. Following enzymatic hydrolysis of the urinary steroid glucosiduronates, the extracts are treated with chromic acid under mild conditions to form androstane-3,11,17-trione and its isomer etiocholane-3,11,17-trione (referred to as "triones" in the text). These "triones" are isolated by paper chromatography and measured quantitatively by the Zimmermann reaction.

METHODOLOGY

The following methodology was developed and applied to 24-hour urine specimens collected from healthy volunteers and patients with various disorders. In most instances collection accuracy was checked by means of specific gravity and creatinine content. The total 17-ketosteroids were

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simultaneously determined using a modification of the procedure described by Drekter *et al.* (8).

In a 125-ml. Erlenmeyer flask, 100 ml. of the urine specimen and a few drops of caprylic alcohol were boiled for ten minutes. After cooling, sufficient glacial acetic acid was added, to bring the solution to pH 6, followed by 2 ml. of .1N acetate buffer at pH 5.3. The solution then was brought to pH 5.15 with sodium acetate or acetic acid, and 25,000 units of β-glucuronidase (Warner Ketodase) and 1 ml. of toluene were added. The mixture was incubated at 38° C. for seventy-two hours.

The solution was then transferred to a 250-ml. separatory funnel and extracted four times with 25 ml. of methylene chloride by gently shaking the funnel for two minutes each time. The combined extracts were dried over anhydrous sodium sulfate. The solution was transferred to a 300-ml. round-bottom flask with two 25-ml. washings, and the extract was evaporated to dryness *in vacuo*.

To the dried residue were added 15 ml. of glacial acetic acid and 10 ml. of chromic acid solution, consisting of 400 mg. of chromic anhydride dissolved in 2 ml. of water and made up to 200 ml. with glacial acetic acid. The mixture was kept at 250° C. for two hours. After this time 0.1 Gm. of sodium bisulfite dissolved in 50 ml. of water was added and the solution was transferred to a 250-ml. separatory funnel. The solution was extracted four times with 25 ml. of methylene chloride and the combined extracts were taken to dryness *in vacuo*. The dried residue was then carefully transferred to a 1-ml. volumetric flask with small washings of methanol.

A sheet of Whatman #1 filter paper was prepared as described by Savard (9) and impregnated with 50 per cent methanolic propylene glycol. For each analysis five 1-cm. strips were utilized:

Strip 1: 50γ of Standard etiocholanetrione.
Strip 2: 50γ of Standard and 100λ of urine extract.
Strip 3: 100λ of urine extract (for qualitative location of zone)
Strip 4: 100λ of urine extract (for quantitative measurement)
Strip 5: 100λ of urine extract (for quantitative measurement)

The strips were developed in a ligroin-propylene glycol system for approximately twenty hours and the chromatogram was then dried in an oven. Care must be taken not to expose the drying paper chromatogram to any source of ultra-violet radiation, since this has been shown to cause destruction of steroids (10).

Strips 1, 2 and 3 were stained with Zimmermann's reagent by wetting the strip first with 2.5 N alcoholic potassium hydroxide followed by blotting and wetting the strip again with 2 per cent alcoholic *m*-dinitrobenzene. The strip was again blotted and heated in an oven at 70° C. for two minutes. The areas on strips 4 and 5 corresponding to the "trione" spot on strip 3 were cut out and eluted with absolute ethanol. A typical chromatogram is shown in Figure 1. The heavy spot corresponds to the "triones" and the small stained area just preceding was identified as adrenosterone. Frequently there are other spots of varying colors, but they are in small quantity and they do not interfere with the determinations, provided the chromatogram be developed for a sufficiently long time to allow adequate separation.

A piece of filter paper from the chromatogram not containing any steroid was also eluted as a paper blank. The eluates were transferred into three colorimeter tubes (19 mm. $\times$ 150 mm.) and evaporated to dryness on the steam bath. After cooling, to each of the three tubes was added 0.2 ml. of absolute ethanol, 0.2 ml. of 2 per cent ethanolic *m*-dinitrobenzene and 0.2 ml. of 2.5 N ethanolic potassium hydroxide. The tubes were then incubated at 25° C. in a water bath for one hour. At the end of this time 10 ml. of

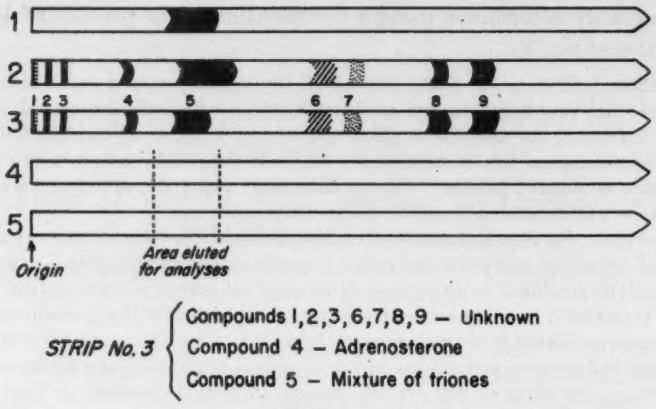

FIGURE 1

95 per cent ethanol is pipetted into each tube. The colorimeter[1] is set at 520 millimicrons, at 100 per cent transmission, and the samples are read[1] as a percentage of the transmission thereafter. The amount of steroid present can be read on a graph by plotting known amounts of steroids against percentage transmission.

VALIDATION OF THE METHOD OF ANALYSIS

Oxidation of model compounds

The optimal conditions for the oxidation of the side-chain of 17-hydroxylated corticosteroids were determined by oxidizing cortisone,[2] cortisol and tetrahydrocortisone for varying periods of time with graded amounts of chromic anhydride. The results of several such oxidations are recorded in Table 1. Five-milligram quantities of the steroids were added to water, followed by extraction with methylene chloride, oxidation, paper chromatography and quantitative measurement with Zimmermann's reagent as described.

Because of the possibility of interfering substances in hydrolyzed urine, similar experiments were conducted substituting urine specimens from patients with hypophyseal insufficiency not receiving corticosteroid therapy. These specimens were shown to contain less than 20γ of 17-ketogenic steroid.

[1] A Coleman Jr. colorimeter was used with a standard of etiocholanetrione (obtainable from U.S.P. Reference Standards). The chromogenic values are the same for both "triones." The standard used to determine recovery of "triones" for Tables 1 and 2 was adrenosterone (Merck), which has a chromogenic value nearly the same as etiocholanetrione. The values obtained are expressed directly as "triones," and are uncorrected for changes in molecular weight during oxidation.

[2] The authors gratefully acknowledge the generous supply of cortisone and adrenosterone from Merck and Company.

TABLE 1. RECOVERY OF CORTICOSTEROIDS AS "TRIONES" AFTER EXTRACTION FROM WATER FOLLOWED BY CHROMIC-ACID OXIDATION

CrO_3 (mg.)	Recovery in 1 hour		Recovery in 2 hours		Recovery in 3 hours		Recovery in 5 hours	
	mg.	%	mg.	%	mg.	%	mg.	%
5 mg. Cortisone Added to 100 ml. of Water								
5.0	3.61	71	3.95	79	4.40	80	4.13	82
10.0	8.23	82	8.07	80	7.93	80	8.07	81
20.0	14.8	74	15.1	76	14.9	74	14.8	74
5 mg. Cortisol Added to 100 ml. of Water								
0.5	.042	8	.061	12	.071	14		
1.0	.110	11	.128	13	.154	15	.253	25
2.0	.438	22	.483	24	.456	23		
3.5	1.48	42	1.57	45	1.68	48		
5.0	3.26	65	3.58	72	3.56	72	3.45	69
10.0	5.93	59	7.05	71	7.44	74	7.03	70
20.0	15.6	78	16.4	82	16.1	81	15.4	77

Quantities of steroid ranging from 2 to 5 mg. were added to urine and the extracts were treated as described previously. The results are shown in Table 2. Optimal yields of about 74 per cent of the "triones" were obtained for oxidation times from two to four hours and with 8 to 20 mg. of oxidant.

Further determinations of optimal conditions were made on 3 patients excreting from 2 mg. to 25 mg. of corticosteroids per day. In all instances maximal readings were obtained by oxidation with 20 mg. of chromic

TABLE 2. RECOVERY FROM 100 ML. OF URINE OF CORTICOSTEROIDS AS "TRIONES" AFTER EXTRACTION AND 2-HOUR OXIDATION WITH CHROMIC ACID

CrO_3 (mg.)	Cortisone				Cortisol				Tetrahydrocortisone	
	2 mg.		2.5 mg.		5 mg.		2 mg.		2 mg.	
	Mg. recovered	% recovered	Mg. recovered	% recovered	Mg. recovered	% recovered	Mg. recovered	% recovered	Mg. recovered	% recovered
2.0									.58	29
4.0									.60	30
8.0									1.49	74
10.0	1.52	75	1.74	70	3.71	74	1.29	64	1.48	74
12.0									1.44	72
20.0					3.65	73			1.41	71

TABLE 3. EFFECT OF OXIDATION TIME AND CHROMIC-ACID CONCENTRATION ON THE YIELD OF URINARY 11-OXYGENATED 17-KETOSTEROIDS

Urine specimens	Urine vol. (ml.)	CrO_3 (mg.)	Yield of urinary 11-oxygenated 17-KS (mg./24 hrs.) in		
			2 hrs.	3 hrs.	4 hrs.
1	1935	10	—	1.6	1.2
1	1935	20	2.6	2.2	0.9
1	1935	30	1.6	—	—
2	2800	10	8.7	8.6	7.5
2	2800	20	15.6	8.9	5.1
2	2800	30	11.7	13.0	8.2
3	2840	10	3.7	3.4	8.2
3	2840	20	25.2	25.2	5.1
3	2840	30	6.6	6.0	—

TABLE 4. ANALYSIS OF 100-ML. ALIQUOTS FROM SINGLE URINE SPECIMENS FOR 11-OXYGENATED 17-KETOGENIC STEROIDS

Specimen	Urine vol. (ml.)	Per analysis (mg./24 hrs.)	Average (mg./24 hrs.)
1	2005	6.8; 6.9	6.9
2	665	2.1; 2.5; 2.4	2.3
3	1300	1.9; 2.1; 2.1; 1.8	2.0
4	775	4.7; 4.5; 4.8; 5.3	4.8
5	890	10.6; 9.9; 9.9	10.1
6	775	2.8; 2.4; 2.1; 2.2	2.4
6	665	6.1; 6.1; 6.4	6.2
7	1090	1.5; 1.6; 1.9	1.6
8	2470	11.9; 11.8; 10.0	11.5
9	985	1.8; 1.8	1.8
10	700	1.9; 1.8	1.8
10	520	2.7; 2.8	2.8
10	1300	1.6; 1.7	1.7
10	1440	0.7; 0.8	0.8
10	930	1.9; 1.2	1.5
11	1160	8.8; 7.3	8.0
12	1640	1.2; 1.3	1.3
12	1615	2.8; 3.0	2.9
13	760	1.0; 0.7; 1.3	1.0

anhydride for two hours at 25° C. as shown in Table 3. These results represent a range of 0.1 mg. to 1.0 mg. of steroids per 100 ml. of urine.

Reproducibility

Serial aliquots of a variety of urine specimens were tested with a satisfactory reproducibility in most instances, both in the high and low ranges of steroid content (Table 4).

During the course of this study it was possible to observe the "trione" excretion of 19 normal subjects (Table 5)—in 5 of them, over a period of several months (Table 6). The normal range of values appears to be 3.9 ± 2.3 mg. per twenty-four hours.

No direct correlation exists between 17-ketosteroid and "trione" values, although in most instances subjects excreting larger quantities of 17-ketosteroids also showed high "trione" excretion (Table 5, Subjects 5, 10 and 14).

As shown in Table 6, the large fluctuations in 17-ketosteroid excretion

TABLE 5. COMPARISON OF 17-KETOSTEROID AND "TRIONE" EXCRETION IN NORMAL SUBJECTS

Subject No.	Age and sex	17-KS (mg./24 hrs.)	Triones (mg./24 hrs.)
4	25 M	10.1	4.9
5	27 M	18.0	10.1
6	25 M	4.8	3.9
7	26 M	10.1	2.2
8	M	8.6	2.2
9	21 M	12.3	2.4
10	M	16.7	8.0
11	M	7.0	4.9
12	35 M	9.9	2.7
13	44 M	11.1	1.3
14	44 M	11.1	5.1
15	33 M	7.1	2.0
16	23 F	17.6	1.6
17	25 F	4.0	1.8
18	30 F	9.1	3.3
19	30 F	9.5	4.4
20	21 F	13.3	5.1
21	24 F	22.2	5.2
22	21 F	8.2	2.9
		M = *11.7*	*M* = *3.9*
		SD = ±*5.5*	*SD* = ±*2.3*

TABLE 6. LONG-TERM STEROID EXCRETION VALUES IN INDIVIDUAL NORMAL SUBJECTS

Subject No.	Date	Urinary creatinine (Gm./24 hrs.)	17-KS (mg./24 hrs.)	Triones (mg./24 hrs.)	Triones	
					Mean	S.D.
12	Nov. 19, '54	2.0	9.9	2.7		
12	Dec. 3, '54	1.7	10.3	3.0		
12	Dec. 16, '54	1.7	9.0	2.0		
12	Jan. 8, '55	1.7	6.7	1.6		
12	Jan. 14, '55	1.9	10.6	3.1		
12	Jan. 21, '55	.9	6.3	2.9		
12	Mar. 10, '55	1.7	9.7	1.9	2.4	± .6
13	Nov. 23, '54	2.1	11.1	2.1		
13	Dec. 8, '54	2.8	12.1	2.8		
13	Dec. 14, '54	2.1	9.2	2.1		
13	Mar. 15, '55	2.4	5.2	2.4		
13	Mar. 8, '55	2.8	5.6	2.8	2.4	± .3
14	Nov. 9, '54	1.6	23.1	3.6		
14	Dec. 3, '54	1.5	21.2	2.2		
14	Dec. 16, '54	1.6	8.7	5.9		
14	Dec. 17, '54	1.6	13.8	3.7		
14	Dec. 30, '54	1.5	19.6	8.1		
14	Jan. 11, '55	1.6	17.3	7.5		
14	Jan. 14, '55	1.4	23.3	4.1	5.2	±2.0
17	Nov. 26, '54	1.1	7.3	1.2		
17	Dec. 10, '54	1.0	8.3	.8		
17	Dec. 23, '54	1.3	9.1	3.3		
17	Jan. 7, '55	1.2	7.0	4.6		
17	Mar. 26, '55	1.1	6.1	4.3	2.8	±1.5
18	Nov. 22, '54	1.5	9.5	4.4		
18	Nov. 29, '54	1.3	8.7	1.0		
18	Jan. 4, '55	1.2	9.4	3.2		
18	Jan. 10, '55	1.4	8.8	2.9		
18	Jan. 15, '55	1.3	6.6	1.7		
18	Jan. 16, '55	1.3	6.3	2.2		
18	Mar. 7, '55	1.3	10.9	2.3	2.5	±1.0
				Total	*3.1*	*±1.7*

in a given subject generally did not parallel either an increase or a decrease in 11-oxysteroid excretion. In some instances there appeared to be a pronounced divergence between the two assays. Measurements of steroid excretion in Subjects 13, 14 and 18 on successive days showed such divergence, and might indicate a differential excretion rate of these two types of steroids.

In both sexes a standard dose of 10 units of long-acting corticotropin (Zinc Cortrophin, Organon) resulted in a marked increase in urinary 11-oxygenated 17-ketogenic steroids (Table 7). Administration of 80 units per day of intermediate-acting corticotropin (ACTHar gel, Armour) to a patient with bronchial asthma resulted in an exceptionally large increase in adrenal steroid production. In patients with anterior pituitary insufficiency

TABLE 7. EFFECT OF CORTICOTROPIN AND CORTISONE ON EXCRETION OF 17-KETOSTEROIDS AND "TRIONES"

Subject	Sex	17-KS (mg./24 hrs.)	Triones (mg./24 hrs.)	Treatment	Comments
1	M	10.1	4.9		Normal
		18.5	13.7	10 U. corticotropin*	
2	M	4.8	3.9		Normal
		10.1	6.9	10 U. corticotropin*	
3	M	10.1	2.2		Normal
		11.6	6.1	10 U. corticotropin*	
4	M	2.4	2.76		Bronchial asthma
		0.41	26.5	80 U. cotricotropin†	
5	F	0.5	0.0		Ant. pit. insuff.
		0.7	2.0	20 U. corticotropin*	
6	M	0.3	0.0		Ant. pit. insuff.
		0.8	2.9	25 mg. cortisone	
7	F	1.6	2.6	10 mg. cortisone	Addison's disease
		2.9	1.8	10 mg. cortisone	
				20 U. corticotropin*	
8	F	0.5	0.0		Ant. pit. insuff.
		0.7	2.0	10 U. corticotropin*	

* Long-acting Zinc Cortrophin (Organon, Inc.).

† Intermediate-acting ACTHar gel (Armour).

there was no detectable corticosteroid excretion until after administration of corticotropin or cortisone. In a patient with Addison's disease maintained on 10 mg. of cortisone acetate (Cortone, Merck) per day there was no increase in the excretion of "triones" after treatment with corticotropin.

DISCUSSION

The use of chromic acid in the determination of adrenocortical steroids has been well documented by Wilson and Fairbanks (11) and has also been reported from this laboratory (12, 13).

The advantages of the assay procedure described here are: 1) its relative simplicity combined with a fairly high degree of chemical specificity regarding the nature of the substances analyzed, 2) analyses of aliquots of the same urine specimens can be adequately reproduced (Table 4), and 3) analytic results are well correlated with the endocrine status of human subjects, as well as with the effects of administration of cortisone or corticotropin. Although somewhat low recoveries (75–80 per cent) of steroids added to urine have been noted using this technique, a fairly good correlation exists between the values obtained in normal subjects during the last three years with our procedure and those reported by Silber and Porter (4) (6.6 ± 3.7 mg. per twenty-four hours) and by Baggett *et al.* (5) (4.5 ± 1.7 mg. per twenty-four hours).

An interesting correlation also exists between our method and that reported by Wilson and Fairbanks (11). Although the latter is a micromethod, maximal oxidation was obtained at the same percentage levels of chromic acid as reported here.

From the evidence currently available in the literature, the primary 11-oxygenated adrenal steroids excreted in the urine are ring-A saturated 11-oxygenated 17-hydroxy C_{21} steroids, such as tetrahydrocortisone and tetrahydrocortisol, the 11-oxygenated androsterones and etiocholanolones and the C_{21} tetrols and pentols recently isolated by Fukushima *et al.* (14). All of these substances, during chromic acid oxidation, would be expected to yield a mixture of the two "triones" which do not separate on a paper chromatogram.

In some instances it might be desirable to determine ring-A unsaturated compounds of similar nature. This can be done without difficulty by analyzing the section of the chromatogram containing adrenosterone. This steroid travels more slowly than the "triones" and separates readily from them. Such an extension of the assay procedure involves only the preparation of one extra colorimeter tube.

SUMMARY

A method is described for the determination of urinary 11-oxygenated

17-ketogenic steroids, using chromic acid to oxidize the major adrenocortical steroids to isomeric androstane-3,11,17-trione and etiocholane-3, 11,17-trione.

The method is shown to reflect the endocrine status of human subjects, and a tentative range of normal values is presented.

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THE CHANGE IN THYROIDAL I^{131} CONTENT BETWEEN 8 AND 48 HOURS AS AN INDEX OF THYROID ACTIVITY

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THE time course of accumulation and discharge of I^{131} by the thyroids of thyrotoxic subjects differs from that of euthyroid subjects not only in the rate of uptake of the I^{131}, but also in the time of reaching the maximum uptake (Fig. 1) and in the rate of the subsequent release. We have found that the change in thyroidal I^{131} content between 8 and 48 hours after oral ingestion of I^{131} (hereafter called the "8–48 hour thyroidal I^{131} change rate") affords a useful index of the activity of the thyroid gland. We report here a comparison of the 8–48 hour thyroidal change rate, the 8-hour thyroidal uptake and the 24-hour thyroidal uptake, all performed in the same group of patients.

METHODS AND SUBJECTS

"In vivo *counting of the thyroid.* This was carried out with an apparatus in which two lead-cathode Geiger tubes,[1] joined in parallel and with appropriate lead shielding, are placed in front of the patient's neck, 16½ inches away from the skin overlying the thyroid isthmus (1). A dose of 15 microcuries (μc.) was used for each tracer test."

Counting of the urine. This was performed in a container holding 1 liter, with a lead-cathode Geiger tube[2] running through the center of the solution (1).

Subjects. All subjects were untreated. In three-quarters of the patients in the thyrotoxicosis group, the diagnosis subsequently was confirmed by observing their response to therapy with antithyroid drugs; in the rest of this group, the diagnosis was clinically unequivocal. All the subjects were living in New Zealand, where a natural iodine deficiency has been alleviated by iodization of the domestic salt (2).

RESULTS

The 8–48 hour thyroidal I^{131} change rate

From Figure 1 it is apparent that the change in thyroidal I^{131} content between 8 and 48 hours is in the negative direction for the average thyrotoxic subject and in the positive direction for the average euthyroid subject. From Table 1 it is seen that the mean value for the thyrotoxic group

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[1] G.26Pb, 20th Century Electronics, London.

[2] G.10Pb, 20th Century Electronics, London.

TABLE 1

Group	I^{131} content (% dose) of the thyroid			I^{131} change rate in the thyroid	
	8 hours	24 hours	48 hours	8–24 hours	8–48 hours
Thyrotoxic (31 cases)					
Mean	77.0	71.9	63.5	−5.1	−13.5
Range	96 to 39	93 to 26	90 to 13	−27 to +9	−46 to +7
Euthyroid (35 cases)					
Mean	30.1	38.6	39.5	+8.5	+9.4
Range	62 to 8	67 to 12	63 to 12	+23 to +2	+23 to 0

is −13.5 and the mean value for the euthyroid group is +9.4, the difference between the groups being 22.9 points. The t value for this difference is 10.5; thus, with 64 degrees of freedom, $P < 10^{-8}$. This shows that the apparent convergence of the thyrotoxic and euthyroid curves between 8 and 48 hours, as shown in Figure 1, is not due to random variation.

Figure 2 shows the frequency distribution in the two groups, according to the 8–48 hour thyroidal change rates.

The 24-hour thyroidal uptake versus the 8-hour uptake

From Figure 1 it is apparent that the difference between the thyrotoxic and the euthyroid groups is greater at 8 hours than at 24 hours. From Table 1 it is seen that the mean change in the thyroidal I^{131} content between 8 and 24 hours is −5.1 for the thyrotoxic group and +8.5 for the euthyroid group, the difference between the groups being 13.6 points. The t value for this difference is 8.53; thus, with 64 degrees of freedom, $P < 10^{-8}$. This shows that the apparent superiority of the 8-hour over the 24-hour measurement is not due to random variation.

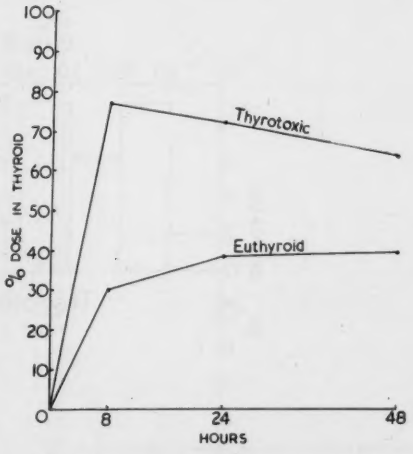

FIG. 1. The change in the I^{131} content of the thyroid (% dose) in 31 thyrotoxic subjects and 35 euthyroid subjects between 0 and 48 hours after oral ingestion of 15 μc. of I^{131}.

The frequency distribution of the two groups according to their 8-hour uptakes of I^{131} is presented in Figure 3, and the frequency distribution of

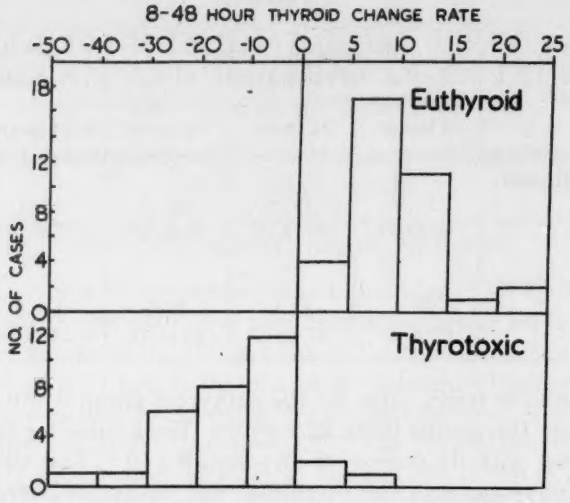

FIG. 2. The frequency distribution of 31 thyrotoxic subjects and 35 euthyroid subjects according to the change in the I^{131} content of the thyroid (% dose) between 8 and 48 hours after oral ingestion of 15 μc. of I^{131}.

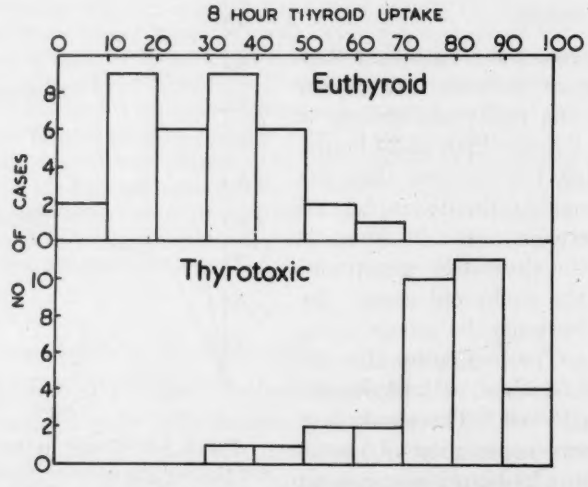

FIG. 3. The frequency distribution of 31 thyrotoxic subjects and 35 euthyroid subjects according to their 8-hour thyroidal I^{131} uptakes (% dose) after oral ingestion of 15 μc. of I^{131}.

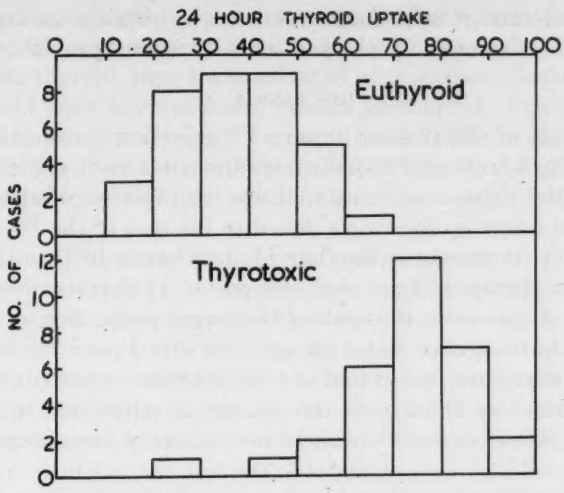

FIG. 4. The frequency distribution of 31 thyrotoxic subjects and 35 euthyroid subjects according to their 24-hour thyroidal I^{131} uptakes (% dose) after oral ingestion of 15 μc. of I^{131}.

the two groups according to their 24-hour uptakes is presented in Figure 4.

The 8–48 hour change rate versus the 8-hour uptake

Figures 2 and 3 show that the 8–48 hour thyroidal I^{131} change rate provides better separation between the thyrotoxic and euthyroid groups. In only 3 thyrotoxic cases did the values overlap the euthyroid range, whereas with the 8-hour thyroidal uptake, there was overlapping in 6 thyrotoxic cases.

Correlation between 8-hour uptake and 8–48 hour change rate

From Figure 5 it is seen that although there is some correlation between these two indices, it is a poor one. Figure 1 shows that both these indices are characteristic in thyro-

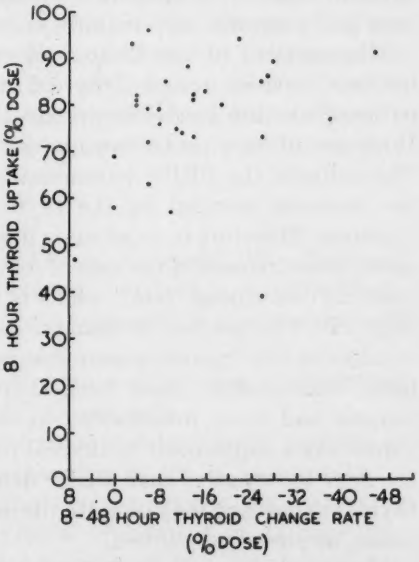

FIG. 5. The correlation between the 8-hour I^{131} uptake of the thyroid and 8–48 hour I^{131} change rate in 31 thyrotoxic patients.

toxicosis. Therefore, it is of diagnostic value to obtain a measurement of both the 8-hour thyroidal uptake and the 8–48 hour thyroidal change rate.

DISCUSSION

Measurement of the 48-hour urinary I^{131} excretion is a worthwhile supplement to the 8-hour and 48-hour measurements, as it provides a check of the thyroidal values, and may indicate the presence of aberrantly situated thyroid tissue by showing a deficit in the sum of the I^{131} excreted in the urine and that present in the thyroid at 48 hours. In 10 euthyroid subjects this sum averaged 92 per cent, whereas in 11 thyrotoxic patients the average was 82 per cent, illustrating the larger proportion of circulating iodine in the latter group. Although agreeing with Fraser (3) that one can differentiate thyrotoxic, euthyroid and hypothyroid cases by urinary measurements alone, we think that the danger of errors due to incomplete collection of urine makes dependence on urinary measurements alone inadvisable.

For differentiating hypothyroid cases from euthyroid cases we find the 48-hour thyroidal I^{131} content the best index.

Measurement of the 8-hour and 48-hour thyroidal I^{131} contents and the 48-hour urinary excretion of I^{131} takes one technician a total time of an hour and a quarter, and requires three 10-minute visits by the patient.

Measurement of the 48-hour plasma protein-bound I^{131} concentration has been used for assessing thyroid function (4), and is useful for assessing patients who are unable to present themselves for *in vivo* measurements. However, urinary measurements can also be used in such circumstances. The value of the PBI^{131} determination depends on the specific activity of the hormone secreted by the thyroid and the rate of secretion of the hormone. Therefore in most cases it would probably be in accord with our direct measurement of the rate of release of I^{131} from the thyroid (the 8–48 hour thyroid change rate), which is dependent on the same two factors. High PBI^{131} values may be found in cases of myxedema (5), so it is desirable to make *in vivo* thyroid measurements to supplement the PBI^{131} determinations. Rather than make both thyroid and PBI^{131} measurements, it is simpler and more informative to obtain 8-hour and 48-hour thyroidal values. As a supplement to thyroid measurements, urinary determinations are more informative than PBI^{131} determinations, as the former check the thyroid values and may indicate the presence of aberrantly situated thyroid tissue, as already described.

Many authors state (3, 5–7) that it is theoretically desirable to have a measure of thyroidal function which is independent of renal function. We think that this is a misconception. If, due to a renal defect, the rate of excretion of iodide by the kidneys becomes reduced, then iodide entering

the blood will remain there for a longer time. If the rate of secretion of thyroid hormone is to remain unchanged without alteration of the iodine content of the thyroid, then the number of micrograms of iodine taken up by the thyroid each day must also remain unchanged. Therefore, as the renal defect is causing a higher average blood iodide level, the rate of clearance of iodide from the blood by the thyroid must be decreased. However, the thyroid must still take up the same percentage of a tracer dose of I^{131}.

It would seem, therefore, that thyroid activity in terms of micrograms of thyroid hormone secreted daily, is estimated more accurately by simple thyroidal I^{131} uptake measurements than by measurements of the rate of thyroidal clearance of plasma I^{131}. The clearance rates are estimates, not of the patient's thyroid hormone secretion rate, but of what it would be were the patient equipped with a hypothetical, standard pair of kidneys.

All I^{131} measurements are affected by the iodine intake. Thus they may be grossly misleading in the absence of any data on I^{127}. When the body is in iodine balance, iodine excretion in the urine will equal (approximately) the body's iodine intake, so an estimation of the amount of I^{127} excreted in the urine in twenty-four hours can be used to make thyroidal I^{131} uptake figures more nearly absolute—the I^{131} figures having given the distribution ratio for iodide between the thyroid and the kidneys. However, I^{127} estimations are difficult and unreliable and, by and large, I^{131} values alone appear to be satisfactorily informative, provided one can be sure that the patient has not been ingesting very large quantities of iodine. Fraser's test for excessive urinary iodide (3) would appear to be a worthwhile precaution.

SUMMARY

The 8-hour thyroidal I^{131} uptake, the 24-hour thyroidal I^{131} uptake and the change in thyroidal I^{131} content between 8 and 48 hours (the "8–48 hour thyroidal I^{131} change rate") have been measured in 35 euthyroid and in 31 thyrotoxic subjects.

The best differentiation between euthyroid and thyrotoxic patients is furnished by the 8–48 hour thyroidal I^{131} change rate.

A better differentiation between euthyroid and thyrotoxic patients is provided by the 8-hour thyroidal I^{131} uptake than by the 24-hour uptake.

For routine assessment of thyroid function we suggest the making of three measurements, *viz:* 1) 8-hour thyroidal I^{131} content, 2) 48-hour thyroidal I^{131} content, and 3) 48-hour urinary excretion of I^{131}. The performance of this procedure takes one technician a total time of an hour and a quarter and requires three 10-minute visits by the patient.

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HYPOTONIA OF THE GALL BLADDER, OF MYXEDEMATOUS ORIGIN

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IT IS well known that myxedematous patients may present hypotonia of the heart (1), of the uterus (2), of the bladder (3), and of the alimentary canal (megaduodenum (4) or megacolon (5, 6)). All signs of hypotonia of the visceral muscles disappear when the patient is treated with desiccated thyroid.

These facts led us to study the tonus of the gall bladder in myxedema. We report here the results obtained in 4 cases of thyroid insufficiency. The absence of clinical and radiologic signs of pituitary disease indicated that the thyroid insufficiency of these patients was not secondary to a deficiency of the anterior hypophysis, but was a primary disorder of the thyroid gland.

METHOD

The tonus of the gall bladder was studied by means of the following technic:

Timed cholecystographic drainage

The method used was that described by Varela Fuentes *et al.* (7). The details are clearly set forth in their article, but in order to facilitate the interpretation of our results, a brief description is given here. The terminology used, both for the standard drainage and for the cholecystographic drainage has been explained by Varela Fuentes and associates in other articles (8, 9).

Description of the technic

The patient is prepared beforehand as for ordinary cholecystography, but receives a double dose of opaque dye—iopanoic acid (Telepaque; Winthrop Laboratories).

Before carrying out the drainage, a radiograph is taken with no duodenal tube in place, similar to the first plate in standard cholecystography.

Duodenal drainage is then established with a Camus semi-rigid tube which can be made to traverse the pylorus rapidly under x-ray control. The patient must be on the x-ray table throughout the examination.

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Once the tube has reached the second portion of the duodenum, all that remains to be done is to record radiographically the changes taking place in the gall bladder during the five successive periods of the drainage.

As many as seven or eight radiographs may be taken, depending on the speed and manner of contraction of the gall bladder.

At the same time, and throughout the examination, we record the nature and the quantity of the bile flowing during the various periods, for which purpose it is measured at two-minute intervals.

The five periods into which the duodenal drainage is divided are as follows:

1. *Period of flow from common bile duct (choledocian time).* This is the time elapsing between the moment the tube enters the second portion of the duodenum and the moment the gall bladder is stimulated. Throughout this time bile normally flows at a rate of 2 to 4 cc. per minute. This period usually lasts from ten to twenty minutes, during which time radiograph No. 2 of the series is taken.

2. *Period of closed sphincter of Oddi.* This is the time during which no bile flows—the period between the introduction of the gall bladder stimulating substance (olive oil) and the resumption of bile flow which announces the reopening of Oddi's sphincter. In this period, x-ray plate No. 3 is taken.

3. *Period of flow of type "A" bile.* This is the time which elapses between the opening of the sphincter of Oddi after stimulation, and the moment that bile from the gall bladder appears. It is determined by macroscopic observation, inasmuch as the "A" bile from the common bile duct and from the cystic duct is very pale in color, contrasting with the intensely dyed gall bladder bile. This period normally lasts only two or three minutes, during which time a 2 to 4 cc. sample of bile is obtained.

4. *Period of flow of gall bladder bile.* This is the time which elapses between the beginning and the end of the flow of gall bladder bile—normally about thirty minutes. A radiograph is taken at the beginning, and one at the end of this period (Nos. 5 and 6).

If the first stimulation fails to make the gall bladder contract (ascertained from the color of the bile and from the radiographs), a second stimulation is carried out with 33 per cent solution of magnesium sulphate. After this, the gall bladder response is observed in the same way as after the first stimulation. The results are recorded in a last radiograph (No. 7).

5. *Hepatic period.* This is the period following the cessation of the flow of gall bladder bile, during which a faintly dyed bile is once more obtained. This is the bile from the hepatic channels. In this type of examination, it is of no importance, and therefore we did not record it.

Once the duodenal drainage has been completed, all the samples of bile obtained are sent to the laboratory, where they are examined microscopically. The bilirubin content of each sample is determined. The data thus obtained serve to confirm the conclusions drawn from the inspection of the bile in the course of drainage.

From the data regarding the quantities of bile measured and the laboratory findings, graphs have been drawn so as to show at a glance the results obtained in each case. The ordinate indicates the volumes of bile obtained, and the abscissa indicates the time taken to obtain these volumes. Faintly dyed bile has been shown in white, medium colored bile in cross-hatching, and darkly dyed bile in black. Below the abscissa are figures which indicate the bilirubin content of the samples corresponding to the various periods.

CASE HISTORIES

The gall bladder was studied by the foregoing technic in 4 cases of primary myxedema. The clinical histories were as follows:

Case No. 1

G. R. (#13708), a 42-year-old woman, was first seen by us on September 22, 1953. Typical primary myxedema had developed during the previous two years. Outstanding manifestations were asthenia, mental sluggishness, infiltrated skin, hypoacusia, hoarseness, pain in the joints, puffy features, and loss of hair. A small nodular goiter was present. There had been a gradual loss of appetite, hyposthenic dyspepsia (a feeling of gastric fulness and heaviness and of protracted digestion), some changes in intestinal motility, halitosis, and frequent nausea on arising.

The basal metabolic rate was −18 per cent, and the blood cholesterol level was 608 mg. per 100 ml.

On September 29, 1953, timed cholecystographic drainage (Figs. 1 and 2) showed fasting hypochlorhydria, hypertonia of the sphincter of Oddi, prolonged drainage time of type "A" bile, and a negative response to the Meltzer-Lyon magnesium sulphate test. After both stimulations, bile flowed very slowly, with greatly reduced volume per minute. The complete graph was long and low (Fig. 2).

Serial cholecystograms (Fig. 1) revealed a large faintly dyed gall bladder. In the last two plates it showed only a slight contraction without emptying, and a slightly denser shadow.

Summary: A hypotonic, non-contractile gall bladder.

On October 2, 1953, treatment was begun with 10 centigrams (about 2 grains) daily of desiccated thyroid, and continued until January 23, 1954. There was marked clinical improvement in the signs of hypothyroidism and in the dyspeptic symptoms.

On January 23, 1954 the basal metabolic rate was −2 per cent, and the blood cholesterol level was 166 mg. per 100 ml.

On January 25, 1954, timed cholecystographic drainage (Figs. 3 and 4) showed fasting hypochlorhydria, a normally functioning sphincter of Oddi, prolonged drainage time of type "A" bile, and a positive response to the Meltzer-Lyon test. Well-concentrated type "B" bile flowed rapidly after both stimulations, with a volume per minute above normal. The complete graph was similar to that for a normal person.

Serial cholecystograms showed a large gall bladder, more opaque than at the previous examination, which responded with a marked contraction at the first stimulation and with complete contraction at the second.

Summary: All abnormalities of the gall bladder found at the time of the first duodenal drainage had disappeared during treatment with desiccated thyroid. The motility of the gall bladder had been restored and there was adequate concentration of bile.

Progress note: Thyroid therapy was discontinued in January 1954 because of tuberculosis of the lung, which was cured following administration of isoniazid and streptomycin. At present, myxedema has recurred, together with the digestive symptoms described.

Case No. 2

J.J.A. (#8) was a 26-year-old woman who was first seen by us on September 28, 1953, as a case of typical myxedema with goiter, dry skin, infiltrated features, headaches, and periods of constipation. Treatment had been almost continuous from the age of $8\frac{1}{2}$ years with 10 centigrams (about 2 grains) of desiccated thyroid daily, but had been suspended for the two and a half years before admission.

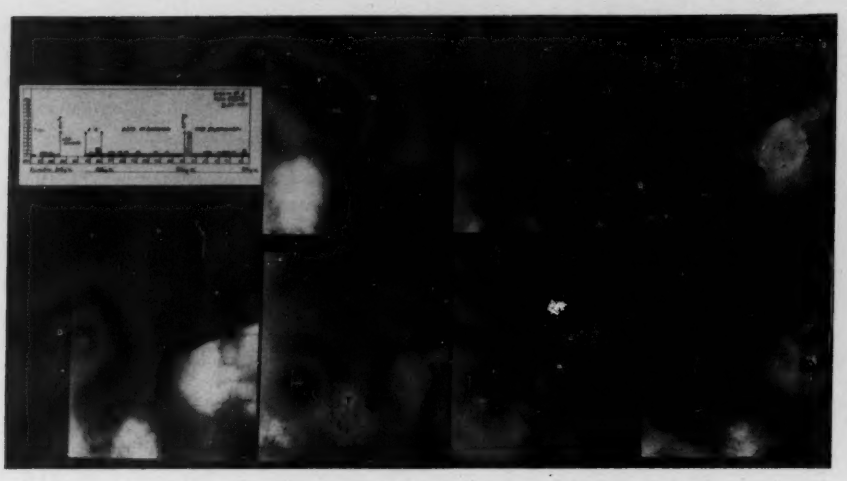

FIGURE 1

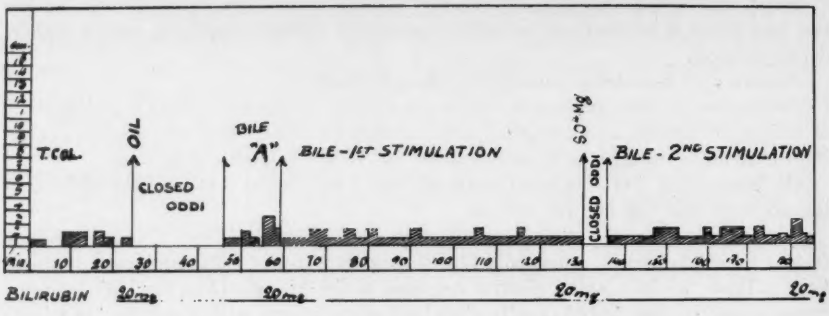

FIGURE 2

The basal metabolic rate was −20 per cent, and the blood cholesterol level was 280 mg. per 100 ml.

On October 2, 1953, timed cholecystographic drainage (Figs. 5 and 6) showed fasting hypochlorhydria, slight hypertonia of the sphincter of Oddi, a positive response to the Meltzer-Lyon magnesium sulphate test, and poor concentration of type "B" bile at the first stimulation (possibly from dilution due to slow emptying of the gall bladder). At the second stimulation, the gall bladder contracted well and bile concentration was good. The slow flow of bile with reduced volume per minute was represented by a long low curve (Fig. 6).

Serial cholecystograms (Fig. 5) showed a well dyed gall bladder of normal shape, which did not contract until the end of the second stimulation.

Summary: Hypotonia of the gall bladder with retarded contraction.

On October 5, 1953, treatment was begun with 5 and 10 centigrams (about 1 and 2 grains) of desiccated thyroid, alternately, per day. By December 24, 1953, there was

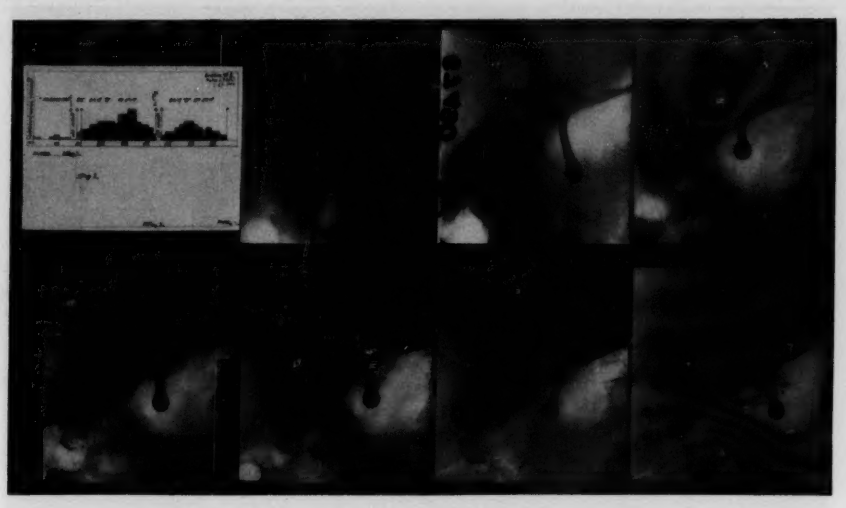

FIGURE 3

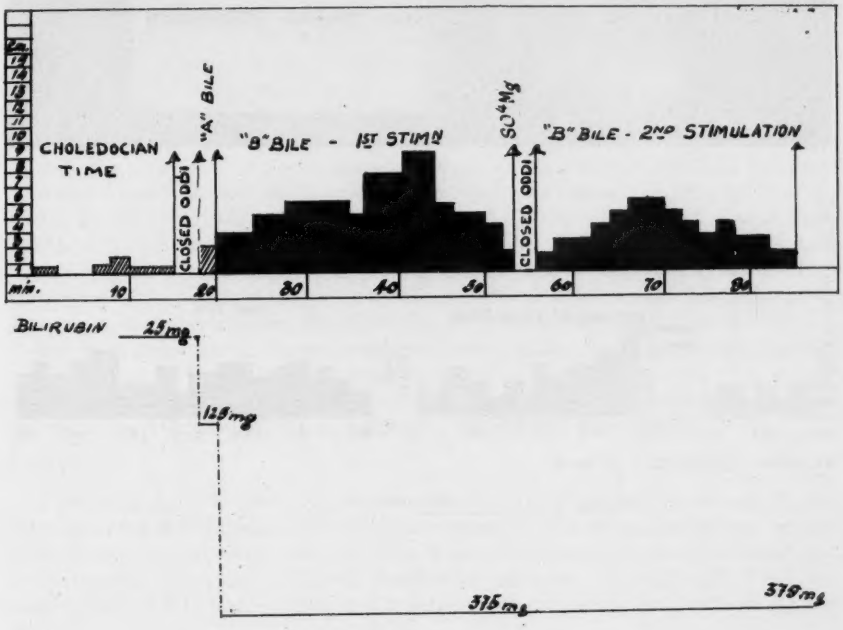

FIGURE 4

FIGURE 5

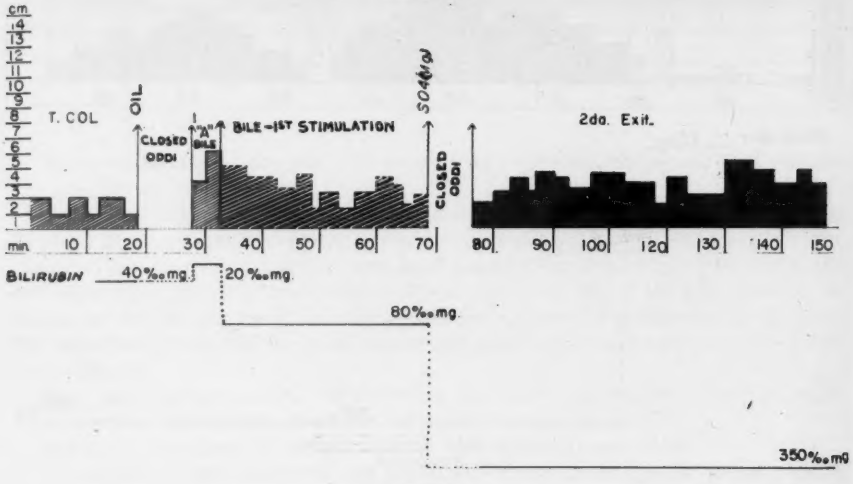

FIGURE 6

definite improvement in the myxedema, and the blood cholesterol level was 149 mg. per 100 ml.

On January 14, 1954, timed cholecystographic drainage (Figs. 7 and 8) showed fasting hypochlorhydria, very slight hypertonia of the sphincter of Oddi, a positive response to the Meltzer-Lyon test, and type "B" bile at the first stimulation. The rate of bile flow (volume per minute) was normal. The complete graph bore a close resemblance to that of a normal person.

Serial cholecystograms showed a well dyed gall bladder of normal shape, with a good contraction at the first stimulation, becoming very intense at the second.

Summary: Duodenal drainage and cholecystography showed that gall bladder activity had returned to normal.

Case No. 3

C. T. (#14549) was a 36-year-old woman with typical primary myxedema. Apparently the onset had been at the age of 6 years. Besides the typical features of myxedema, this patient had hypoasthenic postprandial dyspepsia and selective intolerance to such foods as fats and eggs. No treatment had been given. On December 26, 1953, the basal metabolic rate was −32 per cent and the blood cholesterol level was 327 mg. per 100 ml.

On December 29, 1953, timed cholecystographic duodenal drainage showed fasting hypochlorhydria, normal function of the sphincter of Oddi, prolonged draining time of type "A" bile. There was a positive response to the magnesium sulphate test; the bile was hypoconcentrated type "B," possibly due to dilution as a result of slow emptying of the gall bladder, and the flow was very slow. The complete graph was long and low.

Serial cholecystograms showed a faintly dyed, hypocontractile gall bladder, with contraction beginning at the first stimulation and becoming more evident at the end of the second stimulation—primary hypotonia of the gall bladder.

On January 2, 1954, treatment was begun with 5 centigrams (about 1 grain) of desiccated thyroid daily, increased gradually to 10 and then 15 centigrams per day. This was continued until February 18, 1954. There was great improvement in the signs of myxedema and in all of the digestive symptoms. On February 18, the basal metabolic rate was +4 per cent and the blood cholesterol level was 123 mg. per 100 ml.

On February 22, 1954, a timed cholecystographic drainage showed fasting hypochlorhydria, a normally functioning sphincter of Oddi, normal draining time of type "A" bile, and a positive response to the magnesium sulphate test, with hypoconcentration of type "B" bile as in the previous drainage. The flow was normal in rhythm, with a normal volume per minute. The graph was of normal appearance.

Serial cholecystograms showed good contraction at the first stimulation. Drainage was suspended, as the patient become tired.

Summary: Gall bladder motility was greatly improved with respect to the first drainage, but defective bile concentration was evident on both occasions.

Case No. 4

E.D. de B. (#13241) was a 16-year-old girl with typical primary myxedema. Symptoms appeared at the age of 13. She had been treated with 5 centigrams (about 1 grain) of desiccated thyroid daily until forty-five days before admission, when treatment had been stopped. Symptoms of thyroid insufficiency returned. On August 27, 1953, the basal metabolic rate was −31 per cent and the blood cholesterol level was 355 mg. per 100 ml.

September 4, 1953, a timed cholecystographic drainage showed fasting hypochlorhydria, a normally functioning sphincter of Oddi, prolonged draining time of type "A"

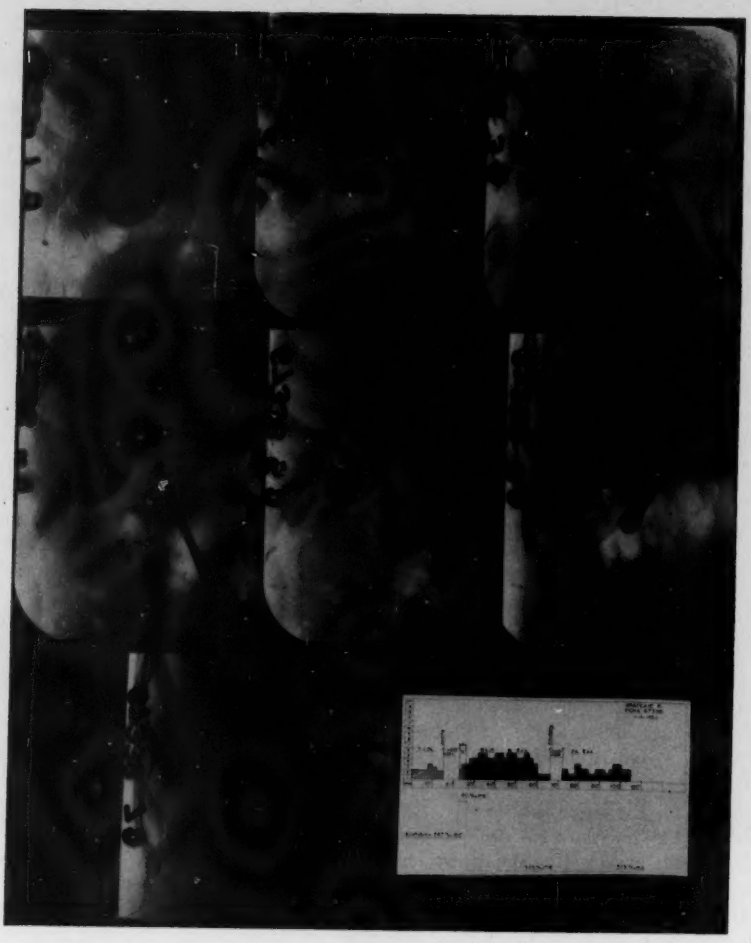

FIGURE 7

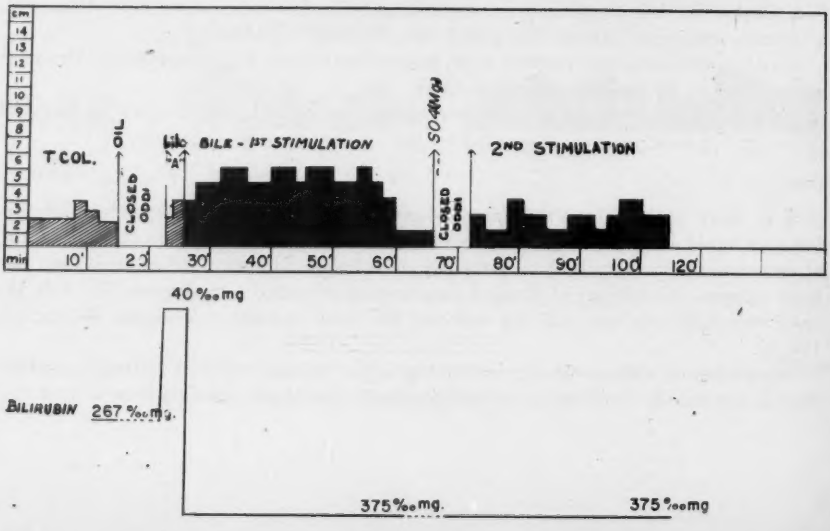

FIGURE 8

bile. There was a positive response to the magnesium sulphate test, with hypoconcentrated type "B" bile, possibly because of dilution due to slow emptying. There was a very slow flow of bile from the gall bladder, with a volume per minute evidently below normal. The complete graph was long and low.

Serial cholecystograms showed a slightly enlarged gall bladder (poorly dyed because of poor concentration) delayed evacuation, and slow contraction beginning at the end of the first stimulation and not becoming evident until the end of the second stimulation. The distention of the gall bladder (visible in the second plate) and the prolonged draining time of type "A" bile suggested the possibility of a cystic syndrome (pain, and a small evacuation of type "B" bile during the flow of type "A" bile).

September 5, 1953, treatment was started with 5 centigrams (about 1 grain) of desiccated thyroid daily. December 15 the basal metabolic rate was −6 per cent and the blood cholesterol level was 160 mg. per 100 ml.

On December 17, 1953, a timed cholecystographic drainage showed a slight hypertension of the sphincter of Oddi. There was a positive response to the magnesium sulphate test, with type "B" bile of greater density than at the previous drainage, but still hypoconcentrated. It was evacuated more rapidly than before, the volume per minute being therefore greater. The complete graph was approximately normal.

Serial cholecystograms showed a slightly enlarged gall bladder, becoming increasingly more opaque, and more opaque than at the previous examination.

Summary: Gall bladder motility was approximately normal at the time of the second drainage but the defect in concentration remained.

DISCUSSION

In our study of 4 patients, the myxedematous origin of the gall bladder hypotonia was indicated by the simultaneous disappearance of the hypotonia and the thyroid insufficiency during treatment with desiccated thyroid.

Simendinger (10) has shown that atony of the gall bladder develops in thyroidectomized dogs. Our observations have shown that there is a similar condition of the gall bladder in the hypothyroid human subject. Thus, it is evident that thyroid function should be studied in all cases of gall bladder hypotonia of unknown origin.

It may be asked whether this hypotonia of the gall bladder can lead to the formation of gall stones in myxedematous patients. In the gall bladders of some of his thyroidectomized dogs, Simendinger found concretions of various sizes composed of pigments, pigment soaps, and traces of cholesterol and calcium; he made similar observations in dogs with biliary stasis produced by mechanical means. It is possible that myxedematous hypotonia of the gall bladder may play a part in the production of gall stones in man. Hypercholesterolemia is apparently not a factor in production, as Simendinger found a normal content of cholesterol in the bile of thyroidectomized dogs, notwithstanding the high levels in their blood. The biliary stasis of myxedema may open up the way to an infectious agent, so often referred to in discussions of the pathogenesis of gall stones.

Two of our patients had hyposthenic dyspepsia. This may have been due to the hypotonia of the gall bladder or to the other digestive disorders frequently associated with thyroid insufficiency, such as hypochlorhydria (11), duodenal hypotonia (4), and hyposecretion of pancreatic enzymes (12). All of these conditions can be corrected by thyroid therapy.

SUMMARY

Four cases are reported of primary myxedema with hypotonia of the gall bladder, studied by timed cholecystographic drainage. That the gall bladder condition was due to thyroid insufficiency was indicated by the fact that the hypotonia and the associated digestive symptoms disappeared after treatment with desiccated thyroid.

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Teaching Clinic

CO-EXISTENCE OF ADDISON'S DISEASE AND THYROTOXICOSIS

SIMULTANEOUS occurrence of Addison's disease and thyrotoxicosis has been reported in 15 cases (1–11). Critical evaluation of these cases, however, reduces the acceptable number to 8. In recent years it has been suggested that hyperthyroidism is by no means uncommon in Addison's disease and that the incidence of thyrotoxicosis in Addison's disease exceeds the incidence in the general population (12), but no case studies have been offered in support of this thesis. In view of the statement regarding the fairly frequent coexistence of the two diseases, and in view of the grave situation which may be caused by their coexistence, as exemplified in the case reported in this paper, it was deemed important to review the subject and to report a case in which the presence of both diseases was amply confirmed.

CASE REPORT

C. R., a 42-year-old white female textile worker was in good health until February 1951, at which time fever, chills and diarrhea developed. About the same time she noticed darkening of the skin, asthenia, anorexia, and weight loss. These symptoms progressed, bouts of nausea and vomiting occurred, and she was admitted to another hospital in June 1951. She had lost 30 pounds in the preceding four months. The examination during this hospitalization revealed hyperpigmentation and hypotension. The urine was normal, the basal metabolic rate was normal, and the hemogram indicated hypochromic anemia. A diagnosis of Addison's disease was made, and treatment with Lipoadrenal extract and desoxycorticosterone acetate was started. For the next seven months her condition improved; asthenia was lessened, appetite and weight increased, and she was able to work part time.

In January 1952, the symptoms of weakness, dyspnea, weight loss and vomiting recurred. She was again hospitalized, and treated for an adrenal crisis. Following her discharge she was treated with larger doses of desoxycorticosterone acetate and Lipoadrenal extract, with very little improvement. She now noticed excessive sweating and increased nervousness. The weight loss continued, and she was unable to resume even part-time employment. In April 1952 another adrenal crisis occurred, and she was again hospitalized.

In May, 1952 the patient was admitted to Jefferson Medical College Hospital. At the time of admission her weight was 118 pounds (her usual weight being 150 pounds). The blood pressure was 110/60, and the pulse rate was 110. She was agitated and emotionally unstable. The only eye sign was stare. Skin pigmentation was increased, and there was pigmentation of the buccal mucosa. The thyroid gland was enlarged. The skin

was warm and moist, and there was tremor of the hands and tongue. She had mild hypochromic anemia. The white blood cell count was 7,700 per cu. mm., and the differential count showed 50 per cent lymphocytes, 12 per cent eosinophils, 2 per cent monocytes, and 36 per cent neutrophils. The urine was normal. Various studies of liver function yielded normal values. With first strength purified protein derivative, the tuberculin reaction was positive; there was a negative reaction to the histoplasmin skin test. The basal metabolic rate was +41 per cent, the serum protein-bound iodine level was 12.6 micrograms per 100 ml., and the 24-hour thyroidal uptake of I^{131} was 65 per cent. The 24-hour urinary excretion of 17-ketosteroids was 1.0 mg., that of the gonadotropins was 26 mouse uterine weight units. The eosinophil count was 267 per cu. mm.; four hours after the intramuscular injection of 25 units of corticotropin it was 318 per cu.mm. An insulin tolerance test (0.1 unit of insulin per kilogram, intravenously) showed the following blood sugar concentrations: fasting, 90 mg.; twenty minutes, 20 mg.; thirty minutes, 20 mg.; and forty minutes, 22 mg. per 100 ml. The test was interrupted forty minutes after the injection of insulin because of the development of coma associated with convulsions. A roentgenogram of the skull was normal. A roentgenogram of the chest revealed a calcific focus at the hilum of the right lung. No calcifications were visualized in roentgenograms of the abdomen. These studies were performed at a time when the patient was receiving no treatment.

Treatment with desoxycorticosterone acetate and Lipoadrenal extract was stopped on the day of admission. Four days later the patient's condition deteriorated; the blood pressure fell to hypotensive levels, nausea and vomiting occurred. A diagnosis of impending adrenal crisis was made. There was an adequate response to treatment with cortisone acetate (50 mg. every six hours), desoxycorticosterone acetate (10 mg. daily) and intravenous infusions of 5 per cent glucose in saline solution. Following recovery from the adrenal crisis, she was treated with propylthiouracil (500 mg. daily), cortisone (50 mg. daily by mouth), and buccal desoxycorticosterone acetate (2 mg. daily). Her response to treatment was excellent. Within a month the pulse rate decreased to 90, she gained 3 pounds, had less tremor, and was less sensitive to heat. The basal metabolic rate was +11 per cent, and the serum protein-bound iodine level was 9 micrograms per 100 ml.

The patient has been followed in the endocrine outpatient department, and has been well. She has regained weight to 148 pounds, and the blood pressure remains within normal limits. She was able to resume full-time work in September 1952, and has continued up to the present time. The dose of cortisone was decreased to her maintenance requirement of 12.5 mg. daily. She continues to take buccal desoxycorticosterone acetate, 2 mg. daily. The dose of propylthiouracil was gradually decreased as the clinical condition improved, and she now takes 50 mg. daily. The serum protein-bound iodine level was 5.6 micrograms per 100 ml. in July 1955. There has been persistence of the slight unilateral exophthalmos which developed in June 1953, one year after the beginning of antithyroid medication, at a time when the thyrotoxicosis was controlled.

DISCUSSION

We have found 15 case reports in the literature in which coexistence of Addison's disease and thyrotoxicosis was believed to have occurred. Most of the cases were observed and reported at a time before newer diagnostic procedures were known and available. Careful evaluation of the clinical picture or autopsy findings has led us to believe that only in 8 cases was the coexistence of the two diseases strongly suggested (1–7). The some-

what superficial similarity of the clinical manifestations of Addison's disease and thyrotoxicosis (hyperpigmentation, weight loss, asthenia, gastro-intestinal symptoms) was discussed in 1928 by Etienne and Richard (13), and could well cause difficulties of differential diagnosis in an era in which reliance had to be placed entirely on the clinical picture. In 7 of the 15 reported cases we believe that the clinical proctocols indicate the presence of thyrotoxicosis, but not of Addison's disease (ref. 3, Case 3; ref. 4, Cases 2 and 3; refs. 8–11). Another report concerns the development of Addison's disease at a time when the thyrotoxicosis was controlled by antithyroid medication (14). Similarly, in at least 4 other cases not included in the 15 previously quoted, Addison's disease developed in patients who had previously suffered from thyrotoxicosis, but at a time when the latter was in complete remission (15–18). These 5 cases are in agreement with Thorn's observation that thyrotoxicosis can be an important factor in initiating Addison's disease (19). Forsham *et al.* (20) observed a patient with Addison's disease in whom a diagnosis of co-existing thyrotoxicosis was suggested by a goiter and a basal metabolic rate of +3 per cent, although the level of serum protein-bound iodine was 3.4 micrograms per 100 ml. A basal metabolic rate in the upper half of the normal range has been thought to be an indication of thyrotoxicosis complicating Addison's disease, because in Addison's disease hypometabolism is usually found (21). However, it would seem hard to accept a diagnosis of thyrotoxicosis if the protein-bound iodine level as well as the basal metabolic rate are within the normal range.

In our case the diagnosis of adrenal insufficiency was suggested by the clinical manifestations of weight loss, anorexia, asthenia, and hyperpigmentation and was confirmed by the low urinary excretion of 17-ketosteroids, the hypoglycemia-unresponsiveness, and the absence of an eosinophilic response to corticotropin. The diagnosis of thyrotoxicosis was suspected because of stare, tremor, tachycardia, warm moist palms, goiter, and emotional instability; this was confirmed by the elevated basal metabolic rate, increased thyroidal uptake of I^{131}, and the elevated level of serum protein-bound iodine. The clinical manifestations of thyrotoxicosis in this patient were no different than those seen in thyrotoxic patients without adrenal insufficiency. The basal metabolic rate, serum protein-bound iodine concentration, and thyroidal accumulation of I^{131} were at levels found in non-addisonian thyrotoxic patients.

These observations in our case and in some of the others reported in the literature would seem to indicate that the changes in clinical signs and symptoms and in various parameters of thyroid function are the same in thyrotoxicosis complicating Addison's disease as they are in thyrotoxicosis not associated with Addison's disease.

The interesting possibility that the typical manifestations of thyro-

toxicosis which developed in this patient were a result of replacement therapy with desoxycorticosterone acetate and Lipoadrenal extract (permissive action?), and might have been less typical if the Addison's disease had remained untreated was considered but not investigated, since the withdrawal of adrenal steroid therapy precipitated an adrenal crisis.

The increased sensitivity of the addisonian patient to thyroid hormone was manifested in this case. At the time her adrenal insufficiency was first diagnosed the basal metabolic rate was normal, and her response to treatment with desoxycorticosterone acetate and Lipoadrenal extract was satisfactory. Seven months after the onset of adrenal insufficiency, thyrotoxicosis developed. The signs of adrenal insufficiency increased, and another adrenal crisis occurred in spite of the fact that at that time she was already receiving increased doses of desoxycorticosterone acetate and Lipoadrenal extract. Conversely, the response of the thyrotoxicosis to treatment with antithyroid drugs was no different in this patient than in patients with uncomplicated thyrotoxicosis. However, as would be anticipated, the management of the Addison's disease presented less difficulty after the thyroid function was depressed to normal levels.

SUMMARY

A case of Addison's disease complicated by thyrotoxicosis is presented. The clinical manifestations as well as the measurements of thyroid function did not differ from those in patients having thyrotoxicosis without adrenal insufficiency. An attempt was made to evaluate critically the cases of co-existence of Addison's disease and thyrotoxicosis reported in the literature. Eight reported cases appear acceptable. The simultaneous presence of both diseases need not materially alter the clinical manifestations of either, but thyrotoxicosis seriously intensifies adrenal insufficiency.

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Letters to the Editor

RENAL BIOPSIES IN CUSHING'S SYNDROME

To the Editor:

During the course of a study of renal morphology in various endocrinologic and metabolic diseases, a search of the literature yielded no clear information about renal structural changes in Cushing's syndrome. Because of this, we have studied surgical renal biopsy specimens taken at the time of adrenalectomy in 2 clear-cut cases of Cushing's syndrome.

In sections of this material, a broadening of the glomerular capillary wall was noted. This lesion is different from any we have previously observed and resembles somewhat, but is not identical with, the swelling in the glomerular tuft in toxemia of pregnancy (1). The delicate, ribbon-like appearance of the capillary wall was noted fairly uniformly in all glomeruli, and was not associated with an increase in PAS-staining material. Slight proximal convoluted tubular "damage" was seen, which was not of the kind reported to occur in association with kaliopenic nephropathy (2, 3). Increased prominence of the juxtaglomerular apparatus was also noted. The glomerular and tubular changes are shown in Figures 1 and 2. Percutaneous renal biopsy was carried out four months after the bilateral adrenalectomy in 1 patient. As shown in Figure 3, the renal structure had partially returned to normal. We are anxious to confirm these findings, but have had great difficulty during the past year in obtaining further cases for study.

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Fig. 1. Section from renal biopsy specimen obtained November 22, 1955, at adrenalectomy for Cushing's syndrome. (Patient H. M., housewife, aged 30 years). Note delicate, ribbon-like broadening or "swelling" of the walls of the glomerular capillaries. There is slight degeneration of the convoluted tubules. Hyaline change can be seen in the wall of the small artery in the lower right-hand corner of the section. (H & E ×330)

FIG. 2. High-power view of a portion of the glomerulus seen in Figure 1. Note the delicate, ribbon-like broadening of the wall of the glomerular capillaries. (H&E ×1100)

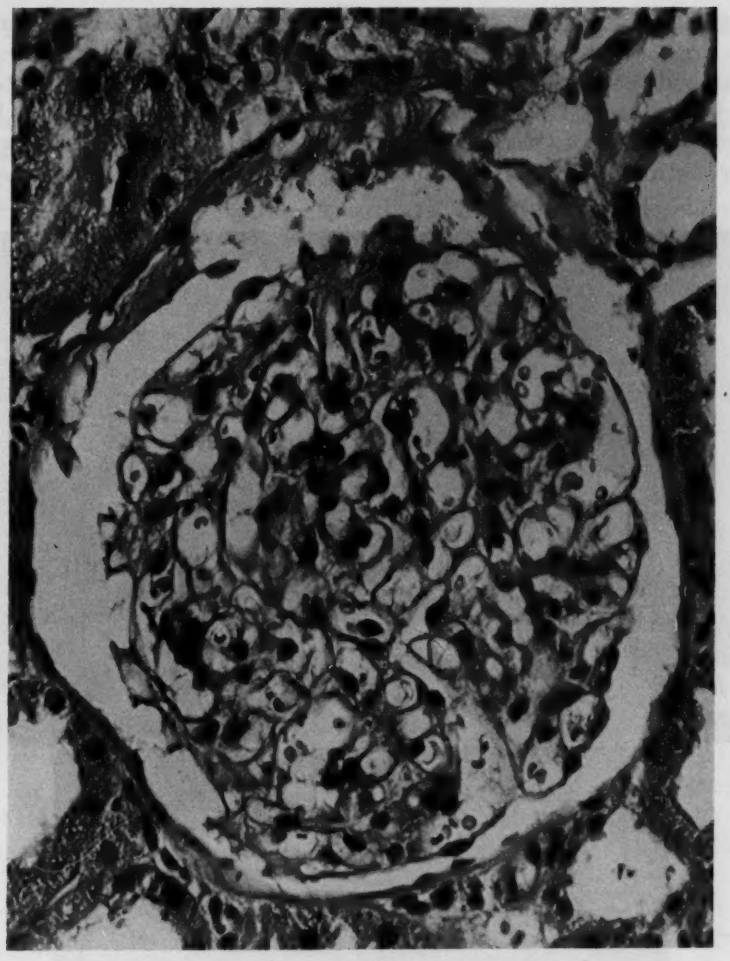

FIG. 3. Section from percutaneous renal biopsy specimen obtained April 19, 1956, four months after adrenalectomy in Patient H.M. Note the nearly normal glomerular tuft and healthier tubules. Proteinaceous material is seen in Bowman's space. (H&E ×500)

SPORADIC GOITROUS CRETINISM IN XERODERMA PIGMENTOSUM

TO THE EDITOR:

The occurrence of hypothyroidism in a case of xeroderma pigmentosum has been reported by Reese and Wilber (1). Recently I have had an opportunity to study the thyroid function in 2 patients with xeroderma pig-

mentosum, a man aged 25 and his sister aged 27 (Fig. 1). Their parents were first cousins. Extreme photosensitivity was evident at the age of 3 weeks and 3 months, respectively. Cutaneous changes characteristic of xeroderma pigmentosum were progressive from early childhood. In both patients the disease became prominent on the head, neck and limbs, where areas of atrophy were found adjacent to hyperkeratoses, acanthotic plaques,

FIG. 1. Showing advanced skin changes present (June 1956) in A, a man aged 25 and B, his sister aged 27. Freckling and atrophy are prominent on the face and neck. The right-sided keratitis of the man has since become re-epithelialized. Moderate thyroid enlargement (B) and extreme hyperkeratosis of the right leg (C) were seen in the woman.

and freckling. Since early adolescence, carcinomas of the skin continued to appear. Both patients were stunted and mentally deficient; they could read but were unable to write. Clinical photographs of the woman at the ages of 13 and 17 showed thyroid enlargement. The weight of her thyroid at age 27 was estimated clinically to be 50 grams. Iodized salt had been used in their household since 1942.

At the age of 21, an exposure keratitis of the right cornea developed in the man, associated with atrophy and entropion of the lower eyelids. Microscopic examination of a fragment of the affected cornea revealed dyskeratosis and hyperkeratosis but no tumor. In December 1955, at the age of 25, he lost his appetite, became sluggish in his activities, and

TABLE 1. THYROID FUNCTION STUDIES

Subject	BMR (%)	Tracer dose of I^{131} (June 12, 1956)		Tracer dose of I^{131} (Aug. 22, 1956)*		Tracer dose of I^{131} (Sept. 17, 1956)	
		24-Hour thyroidal uptake (% dose)	48-Hour serum PBI^{131} (% dose/L.)	24-Hour thyroidal uptake (% dose)	48-Hour serum PBI^{131} (% dose/L.)	24-Hour thyroidal uptake (% dose)	48-Hour serum PBI^{131} (% dose/L.)
Woman aged 27	−27	60	0	33	0	47	0.12
Man aged 25	−33	69	0.039	29	0	71	0

* In both, vitamin A therapy was started June 25, 1956 and stopped August 11, 1956. The man had been treated with vitamin B_{12} since his previous thyroid function studies.

in the opinion of his mother appeared to be following the terminal path of an elder brother who died from xeroderma pigmentosum in 1937. At this time the corneal surface was covered by a keratotic plaque. The hospital ophthalmologist considered that enucleation of the eye would become necessary. The left cornea was affected by a small patch of keratitis, near the limbus.

In the following month (January 1956) he was given each day an oral dose of 50,000 I.U. of vitamin A acetate, and 50 μg. of vitamin B_{12}. Treatment was then stopped. Three months later, the keratitis had disappeared from the left cornea, and the plaque on the right cornea had become smaller. His skin was softer and more moist than that of his sister, and free of acanthotic plaques. He was eating well and gaining weight.

In June 1956 estimations of the basal metabolic rate, thyroidal I^{131} uptake and serum PBI^{131} level (Table 1) indicated that the patients were cretins with an error in the synthesis or release of thyroid hormone.

Both patients were given oral vitamin A, 25,000 I.U. daily, for two months. Vitamin A was then stopped and the I^{131} studies repeated (Table 1). In the subsequent month the man was given a daily oral dose of 25 μg. of vitamin B_{12}. The I^{131} studies were again repeated. Following more

recent vitamin A therapy there has been improvement in the skin of both patients, re-epithelization of the right cornea of the man and regression in the goiter in the woman. The results in Table 1 show that vitamin A depressed the thyroidal I^{131} uptake in both subjects. Withdrawal of vitamin A was followed by an increased uptake in both and by the release of PBI^{131} in the serum of the woman. The effect of vitamin B_{12} was not evident.

Thyroid hormone is required for normal metabolism of vitamin A. Lipsett and Winzler (2) found that the thyroid glands of rats deficient in vitamin A were less able to form organic iodine, although the uptake of iodine was not impaired. Vitamin A is concerned in the nutrition of the skin. The defective sulphur metabolism found in vitamin A deficiency (3) may explain the increased photosensitivity of xeroderma pigmentosum. Further observations will be made of the effects of vitamin A on these patients.

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The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

The Committee on Local Arrangements is Dr. Rulon W. Rawson as Chairman with Drs. Earl T. Engle, Joseph W. Jailer, Warren O. Nelson, and Martin Sonenberg as members of the Committee.

All Scientific Sessions will be held in the Hotel New Yorker. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 31, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the New Yorker, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Eleanor Venning, Royal Victoria Hospital, Montreal, Quebec, Canada, *not later than February 1, 1957. It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that teese will be presented at the meeting*—in order that they may be of reference value and suitable for printing in the program and journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. Reference, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*,

who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter perfect, since there will be no opportunity for proof reading by authors.

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should

be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1957 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Statler Hotel, New York City, on *May 28, 29 and 30, 1957.*

THE VAN METER PRIZE AWARD FOR 1957

The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association which will be held in the Hotel Statler, New York, May 28, 29 and 30, 1957, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, *not later than January 15, 1957.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend. The essay will be published in the annual proceedings of the Association.

GRANTS OF ANTERIOR PITUITARY HORMONES

The Endocrinology Study Section of the National Institutes of Health, Bethesda 14, Maryland, announces a plan for supplying anterior pituitary hormones, other than ACTH, to qualified investigators in the medical sciences. Five of the individual Institutes of the National Institutes of Health are jointly providing the funds for the purchase or production of large uniform lots of the hormones, purified to meet exacting specifications, set by the Study Section, both for potency and for low limits of contamination with other activities.

The first lots of bovine growth hormone and of ovine prolactin have been approved by the Study Section and will be ready for distribution about November 1, 1956. The growth hormone and prolactin are packaged as sterile, lyophilized powders in vials of 50 mg. and 25 mg., respectively. Data on the estimated potency and degree of contamination, and instructions for dissolving the materials, will be issued with each package. A pilot plant at Emory University is being set up under the direction of Drs. Stanley Ellis and Alfred E. Wilhelmi for the production of follicle-stimulating, luteinizing and thyrotrophic hormones. These materials will not be ready for some months.

Details of the program are being looked after by a Subcommittee of present and past members of the Study Section: Drs. Warren O. Nelson, Roy Hertz, Robert W. Bates, and Alfred E. Wilhelmi, Chairman. Dr. Gregory Pincus is Chairman of the Study Section and Dr. Sam R. Hall is its Executive Secretary. In addition, the Subcommittee enjoys the expert advice and services of Drs. W. H. McShan, George Sayers, Paul Munson, C. H. Li, John G. Pierce, and Peter Condliffe, who act as consultants.

Grants of the hormones will be made to qualified investigators who apply to the Endocrinology Study Section. Application may be made by a letter setting forth a brief description of the proposed work, together with an estimate of the amount of hormones required. Requests will be granted to the extent that supplies of the hormones allow.

It is hoped that by this service both clinical and fundamental studies on the anterior pituitary hormones will be stimulated. At the same time, it is thought that much advantage will be gained from the fact that a large number and variety of studies may be made by numerous investigators all using the same highly purified and carefully tested preparations. Continuing support for the program will be sought if, as is thought by the Study Section, work in these fields has been limited by the availability of suitable materials.

NOTICE OF CHANGE OF TITLE AND COVERAGE. INTERNATIONAL ABSTRACTS OF BIOLOGICAL SCIENCES

The Council of Biological and Medical Abstracts Ltd. has decided to change the title of their journal *British Abstracts of Medical Sciences* to *International Abstracts of Biological Sciences.*

This decision results from two major developments. The first is an extension of coverage to a wider range of biological research subjects published in journals throughout the world, and the second is that the International Abstracts of Biological Sciences, with the co-operation of the specialist editors selected by the Institute of Scientific Information of the Academy of Sciences of the U.S.S.R. will include, as from the January 1957 issue, translations of the important Russian papers abstracted in the *Referativny Zhurnal Biologii* (Soviet Biological Abstracts) and *Referativny Zhurnal Biologischekoi Khimii* (Soviet Abstracts of Biological Chemistry), to be published simultaneously with their appearance in Russian.

International Abstracts of Biological Sciences abstracts the world literature in the following fields: Anatomy, Animal Behaviour, Biochemistry, Biophysics, Cytology, Embryology, Endocrinology, Epidemiology, Experimental Biology, Genetics, Haematology, Histochemistry, Histology, Immunology, Microbiology, Nutrition, Odontology, Parasitology, Pathology, Pharmacology, Physical Anthropology, Physiology, Radiation Effects, Toxicology and Viruses.

International Abstracts of Biological Sciences is published for the Council of Biological and Medical Abstracts Ltd. by Pergamon Press, London and New York, on a non profit-making basis. The Honorary Editor is C. C. N. Vass, M.Sc., Ph.D., M.B., Ch.B. On the Council are represented: The Association for the Study of Animal Behaviour (J. D. Carthy, M.A., Ph.D.); The Biochemical Society (R. K. Callow, M.A., D. Phil., B.Sc.) (R. A. Robinson, M.Sc., LL.B., F.R.I.C. (Hon. Sec.)); The Association of Clinical Pathologists (A. G. Signy, M.B., B.S., M.R.C.S., L.R.C.P.); The International Association for Dental Research (A. E. W. Miles, F.D.S., L.R.C.P., M.R.C.S.); The Society for Endocrinology (I. W. Rowlands, M.Sc., Ph.D.); The Society for Experimental Biology (Ed. to be appointed); The Nutrition Society (G. H. Bourne, D.Phil., D.Sc.); The Pharmacological Society (C. A. Keele, M.D., F.R.C.P.); The Physiological Society (H. P. Gilding, M.A., M.D. (Hon. Treasurer)), (W. D. M. Paton, M.A., D.M.), (C. C. N. Vass, M.Sc., Ph.D., M.B., Ch.B.) and (E. B. Verney, F.R.S., F.R.C.P.).

Since the Russian abstracts will have to be translated, the Council re-

quires translators with knowledge of Anatomy, Animal Behaviour, Biochemistry, Endocrinology, Experimental Biology, Experimental Medicine, Microbiology, Odontology, Pathology, Pharmacology and Physiology, and would welcome offers of help. Terms of payment and other details may be obtained from the Honorary Editor: Dr. C. C. N. Vass, c/o Pergamon Press Ltd., 4 & 5, Fitzroy Square, London, W.1, or 122 East 55th Street, New York 22, N.Y.

ACADEMY OF MEDICINE OF CINCINNATI CENTENNIAL

The Academy of Medicine of Cincinnati cordially invites all physicians, their families, and their patients to its 100th Birthday Party, *February 27 through March 5, 1957*. A Health Museum and Exposition will be established in Cincinnati's spacious and historic Music Hall. One hundred and seventy-five health and scientific exhibits, representing medicine, hospitals, research centers, public health, nursing, pharmacy and industry will be displayed.

Dr. Paul D. White and Dr. Walter Alvarez, noted medical scientists and authors, have accepted invitations to be among the distinguished guest speakers.

The Centennial Convocation will be held on the last night of the Exposition, March 5, 1957. The Convocation address will be given by Sir Edward Appleton, Nobel Laureate, Edinburgh, Scotland. Civic leaders, officials of both the American and State Medical Associations, and Government dignitaries will take part in the elaborate ceremonies.

Professor Reginald McGrane, Chairman of the Department of History, University of Cincinnati, has prepared a one hundred year history of the Academy of Medicine, entitled "The Doctor's Forum." Copies of this volume will be available at the Centennial Exposition.

The Cincinnati Journal of Medicine also is preparing a special Centennial Edition for the occasion.

BOOK REGISTER

Ageing in Transient Tissues. Volume 2 of Ciba Foundation Colloquia on Ageing, edited by G. E. W. WOLSTENHOLME and ELAINE C. P. MILLAR; 272 pages; 96 illustrations; 1956. Twenty-seven authorities from both sides of the Atlantic participated in this symposium. Among the subjects covered are: organ culture studies of fetal rat reproductive tracts; the age factor in some prenatal endocrine events; the regenerative capacity of ovarian tissue; the history and fate of redundant follicles; mitochondrial changes in different physiological states; chronological changes in placental function; modifications in the fetal development of the rat after administration of growth hormone; ageing of the axillary apocrine sweat glands in the human female; and ageing in human red cells. Boston; Little, Brown and Company. Price $6.75.

Esterilidad Conyugal. By NORMANDO ARENAS, Profesor adjunto de Ginecologia de la Facultad de Ciencias Médicas de Buenos Aires, Jefe de la Clinica Ginecológica del Hospital Ramos Mejia; ALBERTO E. BETTINOTTI, Secretario de la Sociedad Argentina para el Estudio de la Esterilidad, Jefe del Departamento de Esterilidad del Hospital Ramos Mejía; and EDMUNDO G. MURRAY, Docente libre de Ginecologia de la Facultad de Ciencias Médicas de Buenos Aires, Vicepresidente de la "International Fertility Association"; con la colaboracion de Especialistas Argentinos y Extranjeros; 562 pages; 160 illustrations; 1956. Twenty-five chapters dealing with the social, physiologic, pathologic, clinical, and therapeutic aspects of sterility, including a round-table discussion. Buenos Aires, Editorial Bibliográfica Argentina.

Etiologic Factors in Renal Lithiasis. Edited by ARTHUR J. BUTT, M.D., Butt Medical Research Foundation, Chief, Urological Service, Baptist Hospital, Pensacola, Florida, with a foreword by W. F. BRAASCH, M.D.; Written by 20 experienced investigators, this is a comprehensive and practical survey of basic concepts and newer knowledge concerning the causes of renal lithiasis; 421 pages; numerous illustrations (some in color); 1956. Springfield, Illinois, Charles C Thomas, Publisher. Price $12.50.

Internal Secretions of the Pancreas. Volume IX of Ciba Foundation Colloquia on Endocrinology edited by G. E. W. WOLSTENHOLME and CECILIA M. O'CONNOR; 301 pages; 100 illustrations; 1956. Over 30 participants took part in this symposium which included such subjects as the metabolic effects of alpha-cell destruction; pituitary growth hormone and blood insulin activity; the control of the secretory activity of the islets of Langerhans; fractionation of insulin; the chemical structure of insulin; a concept of the three-dimensional structure of insulin; and glycogen; the action of glucagon on liver phosphorylase; the hepatic action of insulin; some problems of permeability of tissue cells to sugars; and pancreatic islets and growth. Boston; Little, Brown and Company. Price $7.00

Medical Effects of the Atomic Bomb in Japan. Division VIII—Volume 8 National Nuclear Energy Series Manhattan Project Technical Section. Edited by ASHLEY W. OUGHTERSON Clinical Professor of Surgery, Yale University School of Medi-

cine; Attending Surgeon, Grace-New Haven Community Hospital, and SHIELDS WARREN, Pathologist, New England Deaconess Hospital; Professor of Pathology, Harvard Medical School, Boston, Massachusetts; 493 pages; 135 illustrations (one in color); 1956. This authoritative treatise outlines the methods of this large-scale medical research project as well as its statistical findings and their bearing on continuing studies of the effects of damaging radiation on living organisms. Besides providing some of the facts necessary for planning sound national defense, this volume is a valuable contribution to fundamental medical knowledge. New York, McGraw-Hill Book Company, Inc. Price $8.00.

Research Techniques in Schizophrenia. Psychiatric Research Report No. 5 of the American Psychiatric Association. APA Mid-Atlantic Regional Research Conference, Georgetown University School of Medicine, Department of Psychiatry, Washington, D. C., March 9–10, 1956, edited by JACQUES S. GOTTLIEB, M.D., ROBERT A. CLEGHORN, M.D., HAROLD E. HIMWICH, M.D., NATHAN S. KLINE, M.D., WILLIAM T. LHAMON, M.D. and GEORGE SASLOW, M.D. Three symposia covering the following subjects: Mental effects of reduction of ordinary levels of physical stimuli on intact, healthy persons; empirical approach to the study of schizophrenia; application of life table methodology to the study of mental hospital populations; the relationship of the therapist to the outcome of therapy in schizophrenia; and operant conditioning methods applied to research in chronic schizophrenia; 153 pages; 1956. Washington, D. C., American Psychiatric Association. Price $2.00.

Säuglings-Enteritis. By PROF. DR. A. ADAM, Direktor der Universitäts-Kinderklinik Erlangen, mitarbeiter O. H. BRAUN, Erlangen, H. HUNGERLAND, Geissen, G. ILGNER, Erlangen, F. KAUFFMANN, Kopenhagen, H. W. OCKLITZ, Rostock, F. ØRSKOV, Kopenhagen, E. F. SCHMIDT, Rostock, K. SCHREIER, Heidelberg, and R. THURAU, Erlangen; 534 pages; 43 illustrations; 1956. Deals with new ideas regarding the etiology and therapy of the enteritis of infants, new observations on its functional and morphologic nature, the problems of sequelae, and prophylaxis. Stuttgart, Georg Thieme Verlag. New York, Intercontinental Medical Book Corporation. DM 60.– $14.30.

Transactions of the Josiah Macy, Jr., Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Group Processes. Second Conference, October 9–12, 1955, Princeton, N. J. Edited by BERTRAM SCHAFFNER, M.D., University Seminar on Communications, Columbia University, New York. Contents—Social structure among penguins; neonate-mother relationship in goat and man; kinesic analysis of filmed behavior of children; the message "This is play"; 255 pages; 23 illustrations; 1956. Price $3.50.

Nerve Impulse. Fifth Conference, September 20–22, 1954, Princeton, N. J. Edited by DAVID NACHMANSOHN, Professor of Biochemistry, Columbia University College of Physicians and Surgeons, and H. HOUSTON MERRITT, Professor of Neurology, Columbia University College of Physicians and Surgeons, New York. Contents—Brain stem and higher centers; the corticospinal system; properties of nerve impulses; monosynaptic reflex behavior of individual spinal motoneurons; and

some properties of excitable tissue; 256 pages; 82 figures; 1956. Price $4.50.

Neuropharmacology. Second Conference, May 25–27, 1955, Princeton, N. J. Edited by HAROLD A. ABRAMSON, M.D., Assistant Clinical Professor of Physiology, Columbia University College of Physicians and Surgeons, and Research Psychiatrist, Biological Laboratory, Cold Spring Harbor New York. Contents—Lysergic acid diethylamide (LSD) and related compounds; effects of drugs on the behavior of animals and on psychoses of man; research on schizophrenia; experimentally induced psychoses in man; and tolerance to LSD-25 and a theory of psychoses; 328 pages; 26 tables; 85 figures; 1956. Price $4.25.

Polysaccharides in Biology. First Conference, April 27–29, 1955, Princeton, N. J. Edited by GEORG F. SPRINGER, William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia. Contents—Problems of communication (nomenclature); problems of classification; bacterial polysaccharides; and blood group substances; 271 pages; 22 tables; 31 figures; 1956. Price $5.00.

CORRECTION

With reference to the article by A. Laufer and F. G. Sulman—"Estrogenic Leydig-Cell Tumor with Multiple Metastases in a Dog. The Problem of Bisexual Hormone Production by Gonadal Cells," *J. Clin. Endocrinol. & Metab.* **16**: 1151–1162 (Sept.) 1956—the authors wish to state that the data regarding the urinary excretion of estriol, estrone and estradiol are not to be considered as definitive and may be disregarded. Authors' proof reached the editorial office too late to make this correction in the published article.